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Interactions Among Drinking and Ground Water Contaminants on Renal and Hepatic Function (unclassified)

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This project was designed to elaborate on possible effects of monochloroacetate (MCA), maleic acid (MA) and dichloromaleic acid (DCMA) on liver and kidney function. These studies have revealed important interactions between these test compounds and various known hepato- and nephrotoxicants. Some of the interactions were of a potentiative nature and some were antagonistic. In addition, chromate appears to enhance the nephrotoxicity of some of these test compounds, an observation consistent with earlier reports on the interactions of chromate with other substances. All of the studies in this report suggest an important role in the tissue non-protein sulfhydryls.

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29 JUL 1988

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(a) Comprehensive List of the Objectives of the Research Effort.

The overall objectives of this research effort are the same as those listed in the original proposal and in the First Annual Technical Report. That material is reproduced below.

> The intent of this proposal is to examine the effects of selected water pollutants and their interactions with chemicals known to produce liver or kidney damage. The chemicals selected for study are either ground or surface water contaminants or are the by-products of chlorination, and hence are drinking water pollutants. The test compounds were selected on the basis of their potential for actions on the kidney or liver. The standard or reference substances to be used in these studies are known nephrotoxicants or hepato- toxicants. These latter substances have well established sites of action and a great deal is known about their mechanisms of action as well.
>
> The major objective of this research, i.e., to examine the interactions of ground and drinking water pollutants on liver and kidney function, is predicated on the hypothesis that a combination of substances present in threshold or subthreshold concentrations may produce adverse effects on certain biological systems that are not predictable from the actions of those chemical substances tested individually. The possible effects observed might be either potentiation or inhibition of the effects of one or the other test substance. The Specific Aims of the project are:
>
> 1. To examine the effects of certain drinking and ground water pollutants (monochloroacetate, dichloroacetate, dichloromaleate, etc.) on hepatic and renal function in dose-response studies with particular emphasis on low-dose and multiple-dosing protocols.
>
> 2. To examine the effects of selected drinking and ground water pollutants in conjunction with other drinking and ground water pollutants or with substances known to be nephrotoxic and or hepatotoxic (e.g., mercuric chloride, chloroform, hexachlorobutadiene, maleic acid).

The Specific Aims of the last year's work have focused on an examination of the effects of dichloromaleic acid on renal and hepatic function and an examination of possible interactions of this compound with various other nephro- and hepatotoxicants. Also, the effects of monochloroacetate in the rat were examined along with the importance of tissue non-protein sulfhydryl groups in the observed toxicity. These efforts will be discussed as individual projects in the next section of this Report.

(b) Status of the Research Effort.

This Report is organized primarily as five separate research projects each of which is about to be submitted for publication. Hence, in this Status Report, each project will be summarized with the presentation of only the most important data. The complete manuscripts will be sent to the AFOSR as soon as they are submitted for publication.

Project No. 1. Monochloroacetate (MCA) is a known contaminant in various drinking water supplies. The reactivity in vitro of MCA for the sulfhydryl group of either GSH or cysteine has been reported to be much less than that of iodoacetate. The possible role of sulfhydryl inhibition by MCA was an object of this study. The experiments reported here were designed to study the toxicity of MCA focusing on pertubations of liver and kidney function, following oral administration. High doses of MCA cause seizures and slight liver and kidney toxicity.

The studies were conducted in male and female Sprague-Dawley rats. Various doses of MCA were administered orally. As necessary, rats were killed by exsanguination under ether anesthesia, and blood and tissue samples collected. All chemical analyses were performed with standard procedures.

The female rat appeared more sensitive to the acute toxicity of MCA. For example, all male rats survived 188 mg/kg for two days of observation while three of six females died by two days. Deaths often were proceeded by seizures, although no seizures or deaths were observed in the first four to six hours after MCA administration at any dose tested.

In the male rats, MCA increased urine volume and decreased urine osmolality, both in a dose-dependent fashion, with changes occurring only at lethal doses. The concentrations of sodium and potassium in the urine increased the first day after MCA even though urine volume was increased. Glucosuria was not seen. MCA had no effects on urine volume or osmolality in female rats, nor was glucosuria seen. Because of the enhanced mortality among female rats, high doses of MCA were not tested. Plasma GPT activity (a marker for liver function) and BUN concentration (a marker of renal function) were both elevated significantly twenty-four hours after the 470 mg/kg dose in male rats. No effects on GPT or BUN were observed at lower doses in either males or females. When low doses were administered to females for 4 consecutive days (94 mg/kg), no effect on plasma GPT or BUN were observed.

Dichloroacetate and trichloroacetate decreased plasma and tissue concentrations of lactate and plasma glucose concentration. Plasma lactate was significantly increased within three hours after 282 mg/kg dose of MCA (1.81 ± 0.21 vs. 0.73 ± 0.10 umole/ml). Plasma glucose was not altered significantly, although the concentration was generally greater than in controls.

The effects of MCA on non-protein sulfhydryls (NPSH), primarily glutathione (GSH), after exposure to MCA was complex. For males, the dose-response relationship was biphasic with the highest dose causing a significant depletion of hepatic and renal NPSH whereas the lower dose groups had increased hepatic NPSH. The depletion of NPSH in the male rat was significant at one hour after MCA administration with recovery evident by four hours and by twenty-four hours NPSH activity was actually greater than control. In the females, the depletion of hepatic NPSH was of longer duration than in the males, and renal NPSH activity was not affected. Pretreatment of rats with SKF-525A before MCA administration did not alter dramatically the effects of MCA on NPSH activity suggesting that bioactivation of MCA is not necessary for the NPSH-depleting effects.

Project No. 2. In the First Annual Technical Report, some observations on the toxicity of dichloromaleic acid (DCMA) were reported along with a few preliminary observations of possible interactions. The interactions of DCMA with a variety of known nephro- and hepatotoxicants has been the focus of recent studies.

Maleic acid (MA), a known nephrotoxicant in experimental animals, has been studied for possible interactions with its chlorinated derivative, DCMA. These possible interactions have been studied in both male and female rats of the Sprague-Dawley strain. All procedures for handling the animals and chemical analyses were by routine techniques well-established in the literature.

Female rats showed a dose-dependent, but modest glucosuria twenty-four hours after DCMA administration. The response in the male rats was less reproducible, although mild glucosuria also was observed. However, when threshold doses of MA (150 mg/kg) and DCMA (300 mg/kg) were given together, the female rats showed a more than tenfold increase in urinary glucose concentration at both twenty-four and forty-eight hours after administration. On average, the male rats showed less than a doubling of urinary glucose concentration under the same experimental conditions. Similarly, the blood urea nitrogen (BUN) of the female rats rose approximately five to seven fold at twenty-four and forty-eight hours after treatment with MA and DCMA, while the BUN of the male rats rose approximately threefold. This effect on BUN also was observed at earlier times, i.e., at six and twelve hours after administration of the combination of MA and DCMA the female rats showed a greater increase than the male rat. Because DCMA was observed to decrease hepatic but not renal NPSH (see subsequent project) the possible role of NPSH depletion in the MA-DCMA interaction was studied. No differences were observed between male and female rats in the depletion of NPSH by the combination of DCMA and MA.

These data indicate that the female rat is more responsive to the interactive effects of DCMA and MA than the male rat. The mechanism underlying this sex difference is unknown, but does not appear to be related to differential effects on NPSH in kidney or liver tissue.

Project No. 3. Carbon tetrachloride (CCl4) is a known hepatotoxicant. Studies were undertaken to examine the possible interaction of DCMA with carbon tetrachloride. The experiments were conducted with Sprague-Dawley rats as indicated above, and all of the procedures involved were standard.

DCMA alone produced a modest rise in BUN in a dose-dependent manner. Similarly, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), both measures of hepatic damage, were increased in the plasma in a dose-dependent fashion after DCMA. These data taken together suggest modest renal and liver damage after DCMA. DCMA also caused a decrease in hepatic NPSH twenty-four hours after administration, but had no effect on renal NPSH. The effect of DCMA on liver NPSH was observed as early as two hours after administration.

Carbon tetrachloride (1 ml/kg) produced a very large (six to ten fold) rise in liver enzyme activities in the plasma at twenty-four hours. However, when given with DCMA (400 mg/kg) only a relatively modest increase two to three fold) in plasma ALT and AST activities were observed, i.e., the DCMA

SECOND ANNUAL TECHNICAL REPORT

Contract: F49620-86-C-0096

Interactions Among Drinking and Ground Water Contaminants on Renal and Hepatic Function

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antagonized the CCl4-mediated changes by approximately 50%. Interestingly, CCl4 appeared to antagonize the DCMA-mediated depletion of hepatic NPSH and elevation of BUN. The increased BUN caused by DCMA was reduced by 50% when DCMA and CCl4 were administered together, while the reduction in hepatic NPSH was completely antagonized.

These data suggest complex and important interactions of DCMA with a known and efficacious hepatotoxicant. DCMA appears to antagonize the effects of CCl4 on the liver and CCl4 appears to antagonize the effects of DCMA on the kidney. The mechanisms of these complex interactions are under study.

Project No. 4. This project and Project No. 5 are an outgrowth of earlier observations of the Principal Investigator. In previous studies it was demonstrated that potassium dichromate (chromate) enhanced dramatically the effects of a number of nephrotoxicants. Some of these effects were suggestive of a potentiation by chromate. Because chromate appeared to alter the effects of a variety of chemical substances (e.g., mercuric chloride and citrinin), it seemed important to examine the interaction of chromate with the compounds of interest in this study. In Project No. 4 the interaction of chromate with maleic acid (MA) was examined. Again, all studies were conducted in Sprague-Dawley rats and standard procedures were used. Subthreshold doses of chromate (10 mg/kg) and MA (150 mg/kg) were used.

The combination of agents produced an increase in urine volume twenty-four hours after injection that was significantly greater than the sum of the individual effects. In addition, despite the fact that treatment with either MA or chromate alone failed to alter glucose excretion in the urine, when both were injected together there was a significant enhancement of the twenty-four hour urinary glucose excretion. BUN also was examined, but no alterations were detected in this parameter with either agent alone or with the combination. Organic ion transport by renal slices prepared from animals exposed to the combination of MA plus chromate also was affected in an exaggerated manner, i.e., both organic anion and organic cation uptakes were inhibited more than anticipated by the sum of the individual effects. This response is suggestive of a potentiated response.

Project No. 5. Hexachloro-1,3-butadiene (HCBD) is a known nephrotoxicant believed to exert its effect either directly or after the renal metabolism of an HCBD-glutathione conjugate formed in the liver. This halogenated hydrocarbon is a known environmental contaminant and it was of interest to see if chromate could alter the response of the kidney to HCBD. Studies were conducted in Sprague-Dawley rats, using standard procedures.

HCBD alone (50 mg/kg) caused a significant increase in urinary glucose excretion. Similarly, increased urine flow was observed in the animals treated with HCBD alone. No effects on urine flow, glucose excretion, urine osmolality, etc. were observed in animals receiving only chromate (10 mg/kg). One day after treatment with the combination of agents, the decrease in urine osmolality and the increase in urine volume were significantly greater than observed after either agent alone. Although at twenty-four hours no greater effect on urinary glucose excretion was observed after the combination of agents than after the individual compounds, at forty-eight hours post-treatment the combination of chromate and HCBD caused a sustained and exaggerated increase in urinary glucose excretion. Interestingly, although HCBD alone produced a steady and sustained rise in BUN through seventy-two

hours posttreatment, when combined with chromate the increase in BUN was less marked with an apparent antagonism of the HCBD response three days post-treatment. Whatever the mechanism underlying the effect of chromate plus HCBD on renal function, this does not appear to be related to alterations in NPSH content of the liver. Hepatic NPSH fell after HCBD treatment, and this effect was not modified by the combination treatment with chromate and HCBD.

The mechanisms that underlie the interactions of chromate with HCBD or MA are unknown. The studies reported here suggest that chromate enhances the susceptibility of the proximal tubule to MA and HCBD. It is possible that the combination of agents exacerbates damage to the brush border of the proximal tubular cell because chromate exerts an additional, direct effect which is revealed only in cells whose functions are compromised, e.g., by MA or HCBD. Another possible effect of chromate may be to damage the luminal cells to allow easier access of MA or HCBD to the interior of the cell so that their adverse actions can be exerted more readily. This latter explanation is attractive since this mechanism of action of chromate could underlie the effect of this metal to enhance the actions of diverse nephrotoxicants.

Other Studies. The pages immediately following contain summaries of three other projects that are not to the point of completion at this time. Accordingly, since manuscripts will not be forthcoming in the near future (thirty to forty-five days), several figures and tables have also been included which pertain to each of the descriptive materials. Please see see appended materials.

(c) Written Publications.

The following manuscripts are each nearly completed and will be submitted for publication in the next thirty to forty-five days.

(1) Monocloroacetate oral toxicity and depletion of tissue non-protein sulfhydryl groups in male and female rats. Authors: Mary E. Davis and W.O. Berndt. Journal: Fundamental and Applied Toxicology.

(2) Differential alterations in the renal function of male and female rats exposed simultaneously to the combination of maleic acid and its chlorinated derivative, dichloromaleic acid. Authors: W.R. Christenson, M.E. Davis and W.O. Berndt. Journal: Toxicology.

(3) The effect in the rat of the interaction of dichloromaleic acid and carbon tetrachloride on renal and hepatic function. Authors: W.R. Christenson, M.E. Davis and W.O. Berndt. Journal: Toxicology and Applied Pharmacology.

(4) The effect of combined treatment with potassium dichromate and maleic acid on renal function in the rat. Authors: W.R. Christensen, M.E. Davis and W.O. Berndt. Journal: Toxicology Letters.

(5) Interaction of potassium dichromate and the nephrotoxicant hexachloro-1,3-butadiene. Authors: W.R. Christensen, M.E. Davis and W.O. Berndt. Journal: Archives of Toxicology.

(d) Professional Personnel.

W.O. Berndt, Ph.D. - Principal Investigator
M.E. Davis, Ph.D. - Subcontractor
W.R. Christenson, Ph.D. - Postdoctoral Research Associate
K. Johnson, B.S. - Research Technologist

No advance degrees have been awarded related to this project.

With the beginning of the third year of funding, it should be noted that the professional personnel involved in this project will have changed. Both Dr. Christenson and Mr. Johnson have left the laboratory of the PI and two replacement personnel have been recruited and are beginning their work. It is not anticipated that this change in personnel will not significantly disrupt this project, although obviously, some delays must be expected.

(e) Coupling Activities.

Three papers were presented at national meetings related to this work. Material related to the first three publications listed above were presented at the Annual Meeting of the Society of Toxicology in Dallas, Texas in March of 1988.

(f) New Discoveries.

None.

(g) Other Statements, etc.

The studies in this report indicate clearly that the original hypothesis was correct in that important interactions have been revealed among various ground and surface water pollutants on liver and renal function. The potential of these interactions for involvement in human health are yet to be determined, but being able to demonstrate these interactions in a well-controlled laboratory setting suggests the importance may be considerable.

Future experiments are being focused on the importance of non-protein sulfhydryl depletion in the interactive effects. With all of the interactions there seems to be an underlying theme of an important role for sulfhydryl groups in the target organs. An effort will be made in the next phase of this study to examine these activities more fully.

CONTRACT F49620-86-C-0096

APPENDICES

INTERACTION BETWEEN MONOCHLOROACETATE AND CHLOROFORM (75 mg/kg)

EFFECT OF MCA PRETREATMENT ON VINYLIDENE CHLORIDE (200 mg/kg)

EFFECT OF MCA PRETREATMENT ON HCBD TOXICITY

Plasma and Tissue Parameters

MALES

Plasma GPT activity showed a dramatic interaction between $CHCl_3$ and monochloroacetate, which was also dependent upon time after administration. The MCA + $CHCl_3$ group had greatly increased plasma GPT activity at 24 hr after treatment (954 ± 274 SF Units/ml vs. 17 ± 1 for MCA + oil or 21 ± 6 for saline + $CHCl_3$). The results for the MCA + $CHCl_3$ group were variable however they varied from 248 to 1410 SF Units/ml, so the variability is not due to some animals showing an interaction and some not, rather the variability is in the magnitude of the response. The interaction between MCA treatment and time was significant for plasma glucose, and the MCA treated groups had lower glucose concentrations 24 hr, but not 48 hr, after MCA. There were no significant effects on BUN, plasma lactate, liver GSH, and plasma sodium and potassium. Both MCA and time after treatment factors were significant for kidney GSH, and the MCA treated groups showed the rebound increase of GSH concentration reported previously.

FEMALES

Plasma concentrations of sodium, potassium, glucose and lactate were not affected by either MCA, $CHCl_3$ or the combination treatment. The $CHCl_3$ treatment effect did not achieve significance for BUN, however the time factor did and, at 24 hr, the MCA pretreated group had a modest increase of BUN. GPT was also somewhat elevated in this group at 24 hr, however the results were variable (range 26-210 SF Units/ml). No significant effects were found on liver and kidney GSH concentrations, and would not be expected at 24 and 48 hr after treatment. Concentrations of glucose, sodium, and lactate in plasma were not affected by any of the treatments.

Urine Parameters

MALES

There were no significant effects on urine volume however urine osmolality was decreased by 30-35% in the $CHCl_3$-treated groups. The interaction with MCA was not significant, however the time factor was, and the saline pretreated group showed recovery (no longer different from oil controls) the second day after treatment. The failure for urine volume to be increased during output of dilute urine suggests that filtration may be impaired to prevent massive fluid losses.

FEMALES

The time after treatment was the only significant factor for urine volume, and in general all groups excreted larger amounts of urine during the first 24 hr after treatment, compared to the second 24 hr. The $CHCl_3$ treatment factor was significant for urine osmolality, and the treated groups had decreased urine osmolality (particularly the second day after MCA +

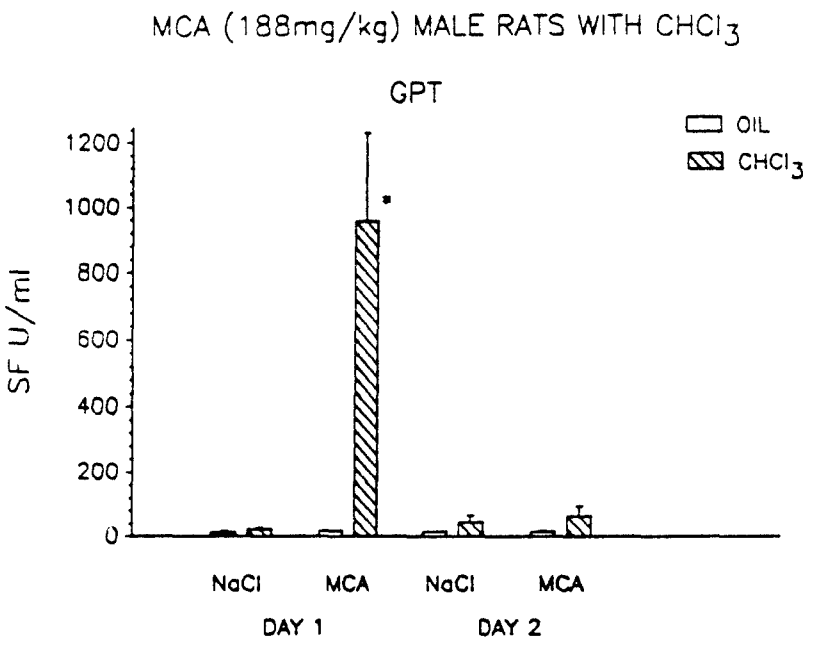
$CHCl_3$), however none of the treated groups were different from their control groups. The sodium concentration in the second day urine was decreased by 64% in the MCA + $CHCl_3$ group and by 57% (not significant) in the saline + $CHCl_3$ groups. The concentration of potassium in urine was decreased in both $CHCl_3$ groups. These support the observation that solute concentration in urine is decreased after $CHCl_3$ and that this decrement is more pronounced after MCA pretreatment.

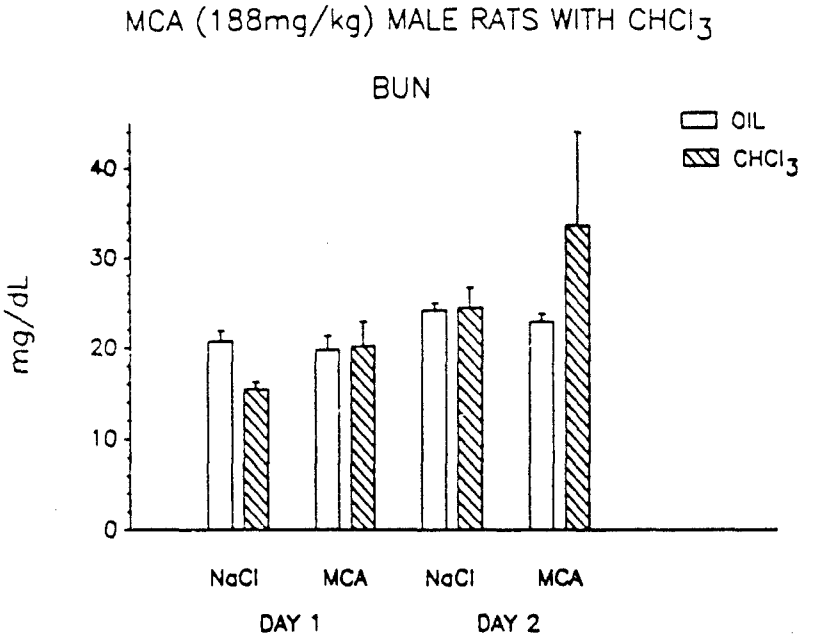
Liver Function

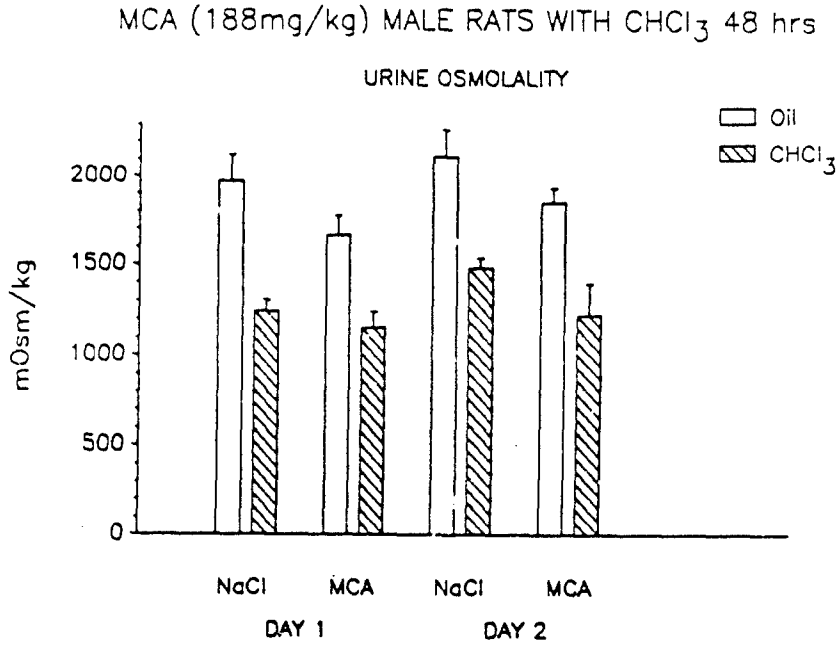
Liver function was studied more fully in female rats to determine if the variable increase of plasma GPT was indicating a subtle hepatotoxicity. $CHCl_3$ treatment decreased the ability to excrete phenolphthalein glucuronide excretion in female rats. This was due in part to a decrease of the bile flow rate, however the concentration of PG in bile was more affected and contributed more to the overall impairment of PG excretion. In this group of experiments MCA itself appeared to decrease bile flow. In the previous clearance study (with VDC) this was seen at 48 hr after MCA, but not 24 hr.

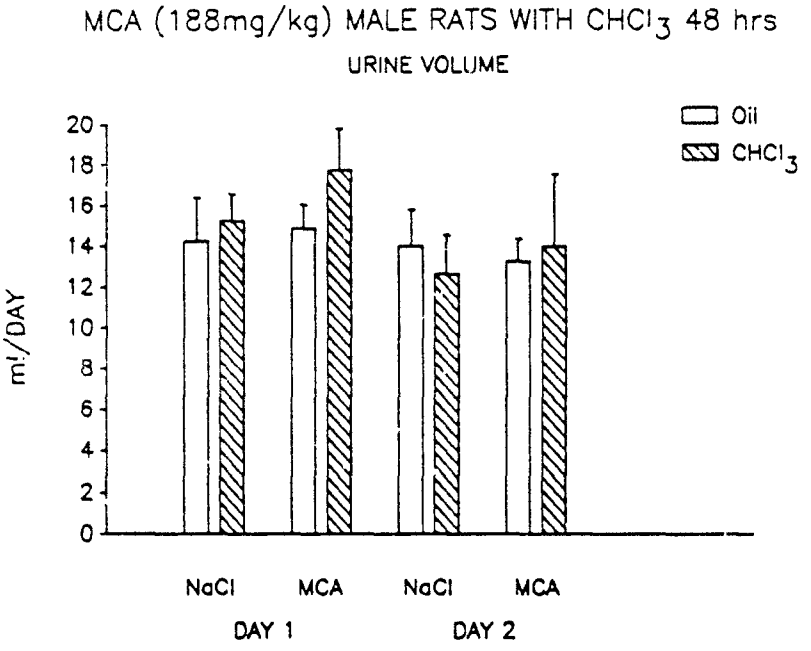
Kidney Function

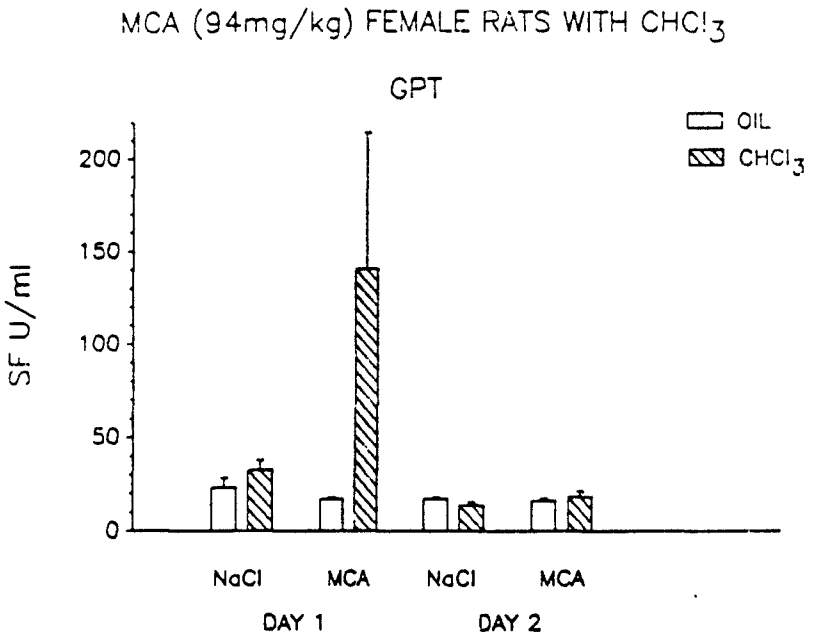
Kidney function also was studied more fully in female rats, to determine the extent of damage underlying the increase of BUN observed at 24 hr in the MCA + $CHCl_3$ group. Both saline and MCA pretreatment groups sustained similar reductions of GFR, assessed as clearance of inulin. The change was more pronounced in the saline group, because the MCA + oil group had a somewhat diminished GFR. There were no significant effects on reabsorption of filtrate or urine flow rate. These results suggest that GFR was decreased by tubulo-glomerular feedback; the macula densa cells sensing the more dilute urine and decreasing filtration at the glomerulus (by constricting the afferent arteriole) to decrease the reabsorptive load for the tubule. This feedback mechanism was successful in matching the load of solute to the ability of the tubule to reabsorb so that only modest changes of urine flow rate and reabsorption occur.

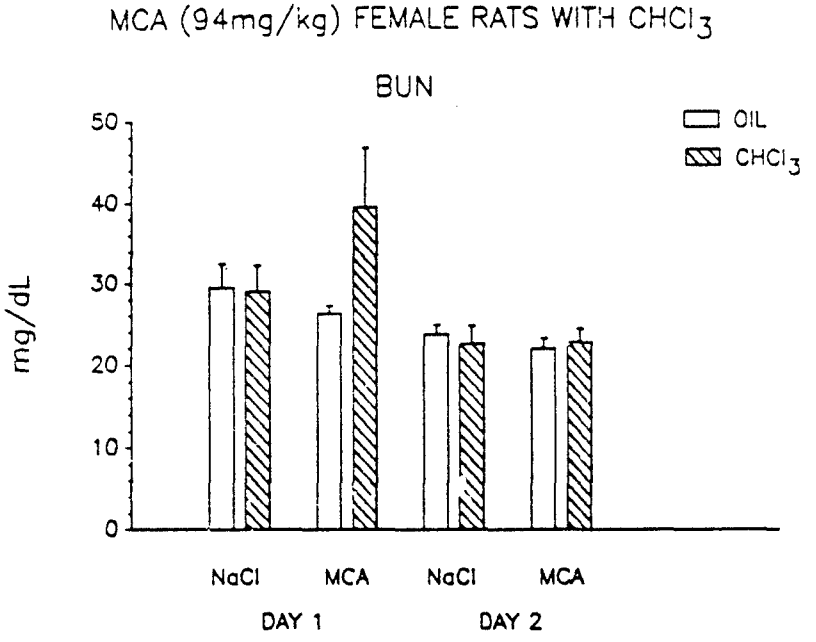

MCA (188mg/kg) MALE RATS WITH $CHCl_3$
GPT
OIL
$CHCl_3$
SF U/ml
1200
1000
800
600
400
200
0
NaCl
MCA
NaCl
MCA
DAY 1
DAY 2

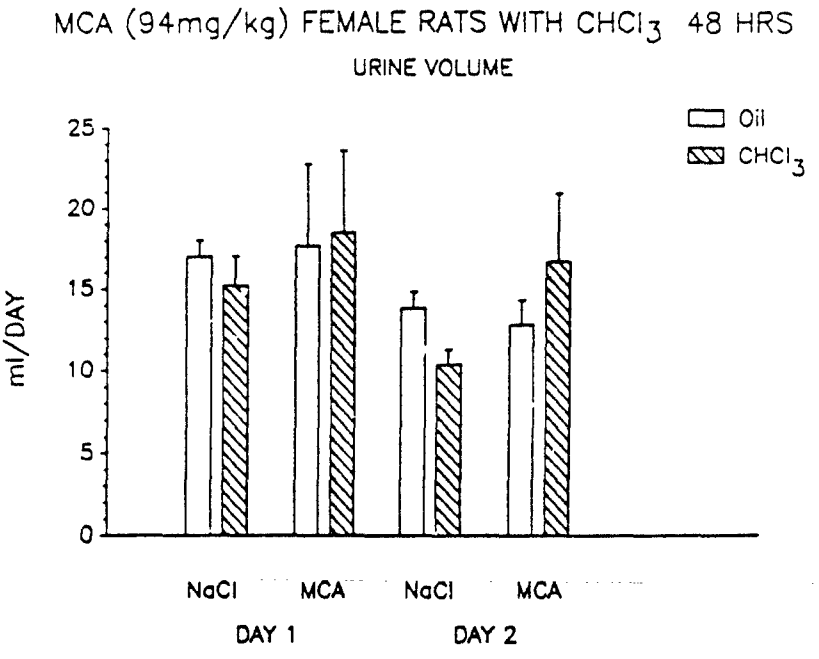

MCA (188mg/kg) MALE RATS WITH $CHCl_3$
BUN
OIL
$CHCl_3$
mg/dL
40
30
20
10
0
NaCl
MCA
NaCl
MCA
DAY 1
DAY 2

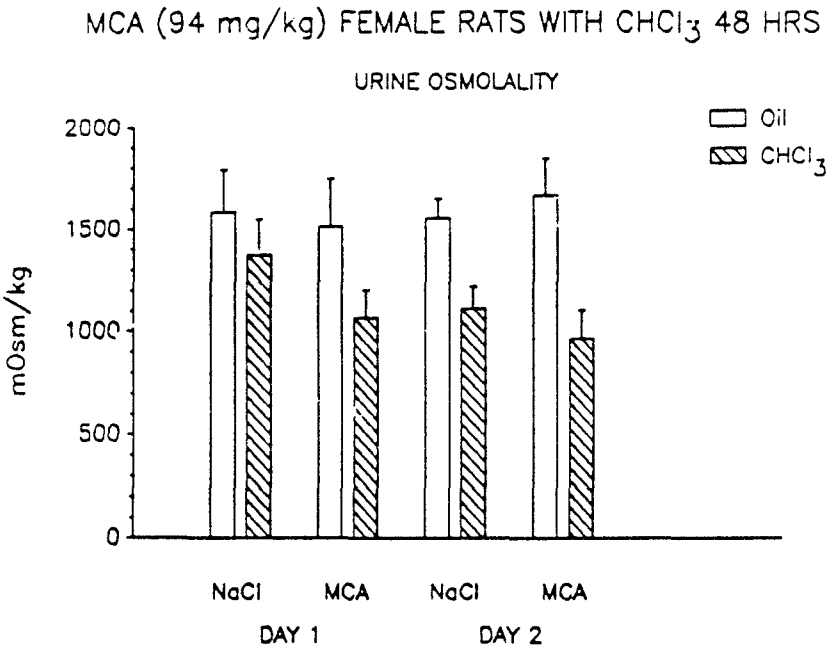

MCA (188mg/kg) MALE RATS WITH $CHCl_3$ 48 hrs
URINE OSMOLALITY
Oil
$CHCl_3$
mOsm/kg
2000
1500
1000
500
0
NaCl
MCA
NaCl
MCA
DAY 1
DAY 2


MCA (188mg/kg) MALE RATS WITH $CHCl_3$ 48 hrs
URINE VOLUME
Oil
$CHCl_3$
ml/DAY
20
18
16
14
12
10
8
6
4
2
0
NaCl
MCA
NaCl
MCA
DAY 1
DAY 2

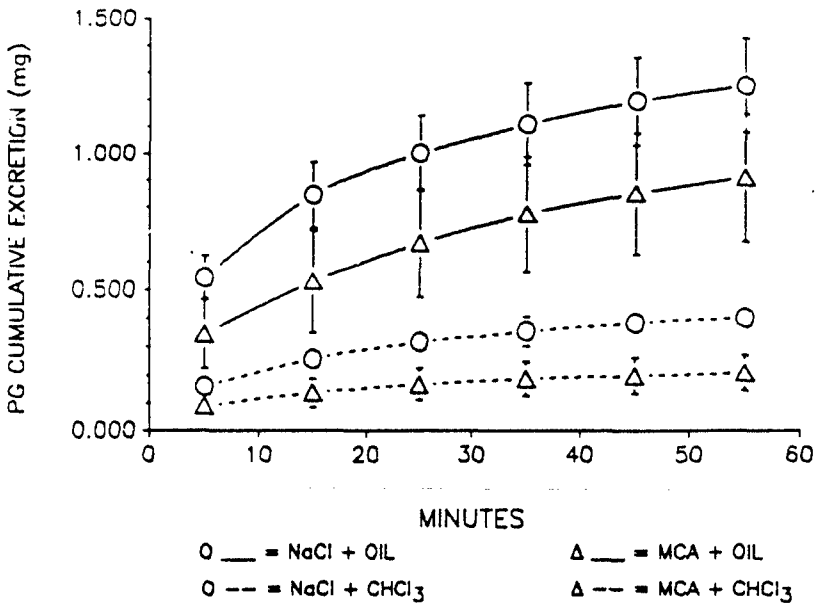

MCA (94mg/kg) FEMALE RATS WITH CHCl3
GPT
OIL
CHCl3
SF U/ml
200
150
100
50
0
NaCl
MCA
NaCl
MCA
DAY 1
DAY 2


MCA (94mg/kg) FEMALE RATS WITH $CHCl_3$
BUN
OIL
$CHCl_3$
mg/dL
50
40
30
20
10
0
NaCl
MCA
NaCl
MCA
DAY 1
DAY 2

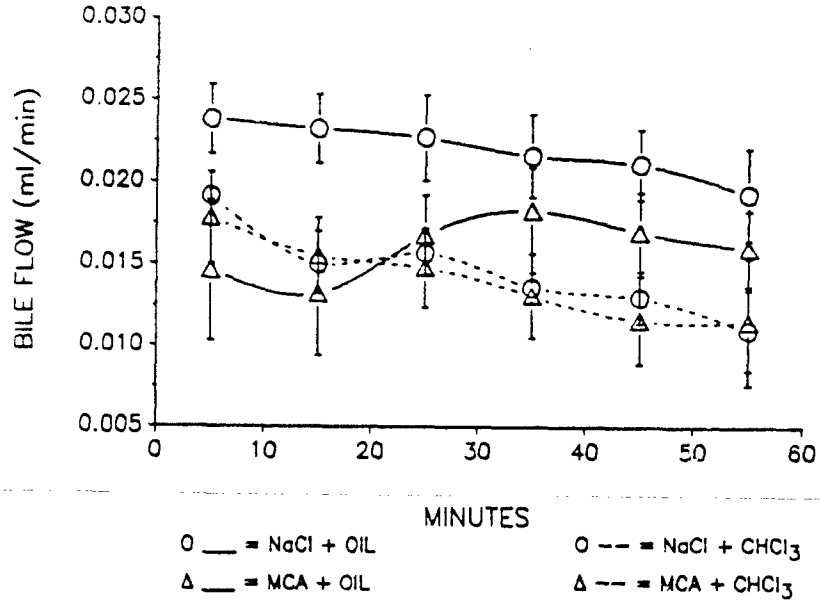

MCA (94mg/kg) FEMALE RATS WITH $CHCl_3$ 48 HRS
URINE VOLUME
Oil
$CHCl_3$
ml/DAY
25
20
15
10
5
0
NaCl
MCA
NaCl
MCA
DAY 1
DAY 2


MCA (94 mg/kg) FEMALE RATS WITH $CHCl_3$ 48 HRS
URINE OSMOLALITY
Oil
$CHCl_3$
2000
1500
1000
500
0
mOsm/kg
NaCl
MCA
NaCl
MCA
DAY 1
DAY 2

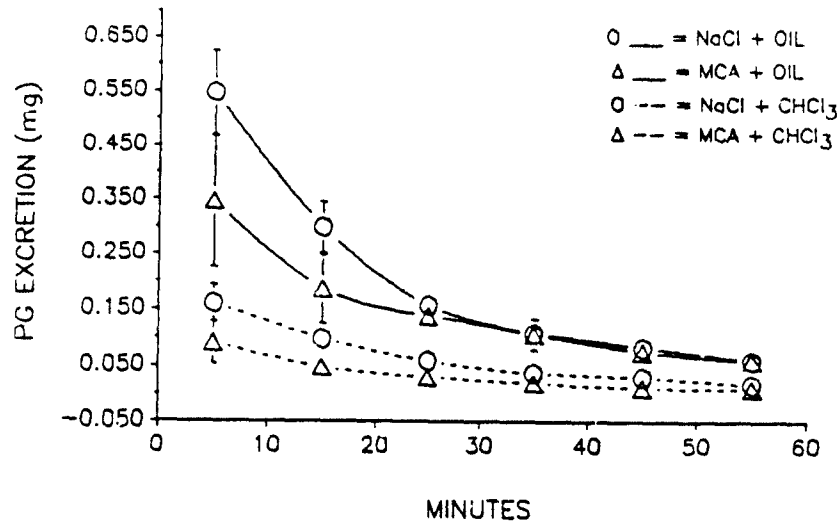
MCA (94mg/kg) FEMALE RATS WITH $CHCl_3$ 24 HRS

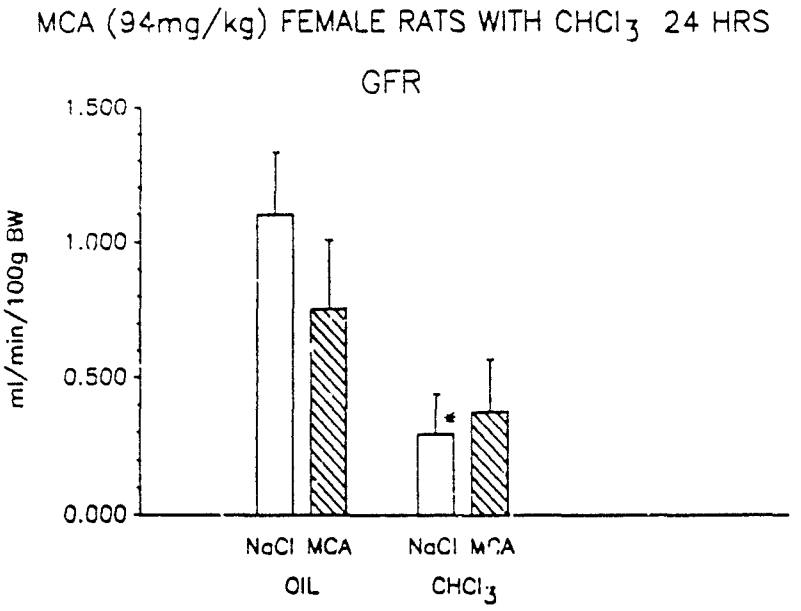


MCA (94mg/kg) FEMALE RATS WITH $CHCl_3$ 24 HRS

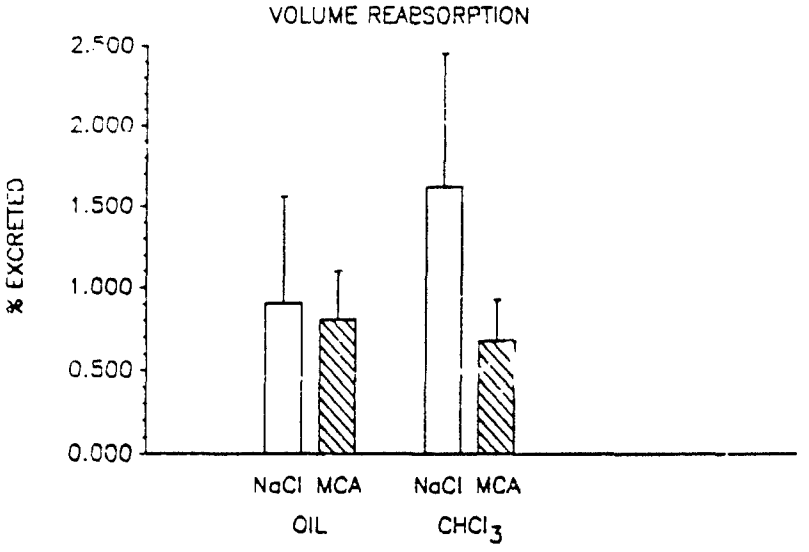


MCA (94gm/kg) FEMALE RATS WITH $CHCl_3$ 24 HRS

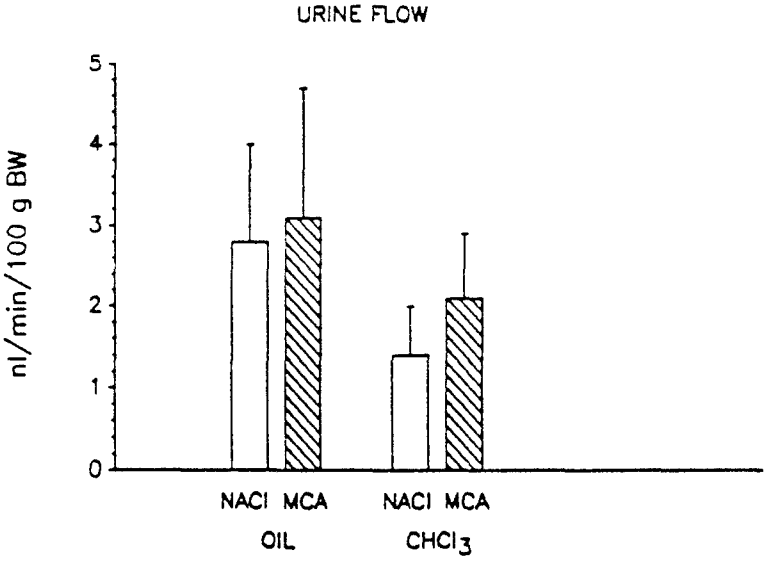



MCA (94mg/kg) FEMALE RATS WITH $CHCl_3$ 24 HRS
GFR
1.500
1.000
0.500
0.000
ml/min/100g BW
NaCl MCA
OIL
NaCl MCA
$CHCl_3$

MCA (94 mg/kg) FEMALE RATS WITH $CHCl_3$ 24 HRS



MCA (94mg/kg) FEMALE RATS WITH $CHCl_3$ 24 HRS



EFFECT OF MCA PRETREATMENT ON VINYLIDENE CHLORIDE (200 mg/kg)

Plasma, Urine and Tissue Parameters

MALES

There were no significant treatment effects on plasma glucose, BUN, GPT, liver or kidney GSH, sodium or potassium. Plasma sodium appeared to be somewhat elevated in the MCA treated groups at 24 hr, but not at 48 hr (and the time factor was significant).

FEMALES

There were no significant effects on plasma glucose, sodium, potassium, GPT activity, liver or kidney GSH. At both 24 and 48 hr after VDC, in both pretreatment groups, the hepatic GSH was somewhat greater in the VDC compared to oil treated controls. This is consistent with an earlier depletion of GSH. BUN was modestly, but significantly elevated in the MCA + VDC group, compared to the MCA + oil group whereas the same dose of VDC given to saline controls did not elevate BUN.

Liver Function

MALES

The interaction between MCA pretreatment and VDC treatment was significant for bile flow at 24 hr, however the differences were slight and no pattern was discernible. There were no effects at 48 hr. Excretion of phenolphthalein glucuronide into bile was unaffected by either MCA or VDC.

Male rats were not affected by the VDC treatment given at the time that hepatic GSH was markedly decreased by MCA pretreatment. Additional males were studied at 6 hr after to VDC, to ascertain that the toxic effect had not been overlooked by sampling at a late time. BUN was not affected by either of the treatments. GPT was increased (262 SF U/ml) in one of the MCA + VDC males, the others were less than controls.

FEMALES

MCA alone did not have any effect on bile flow rate. The effect of VDC to decrease bile flow rate was significant at both 24 and 48 hr, however there was no interaction between pretreatment with MCA and treatment with VDC. Biliary excretion of phenolphthalein glucuronide similarly, and dramatically impaired, at 24 hr after VDC in both VDC groups regardless of pretreatment, and there was not an interaction between MCA and VDC. This is observed if the results are expressed as excretion per 10 minute collection period or cumulative excretion. Females appear to be more sensitive to the effects of VDC on liver function than do males. By 48 hr recovery was evident in both VDC groups, with the saline pretreated group being only slightly reduced.

Kidney Function

MALES

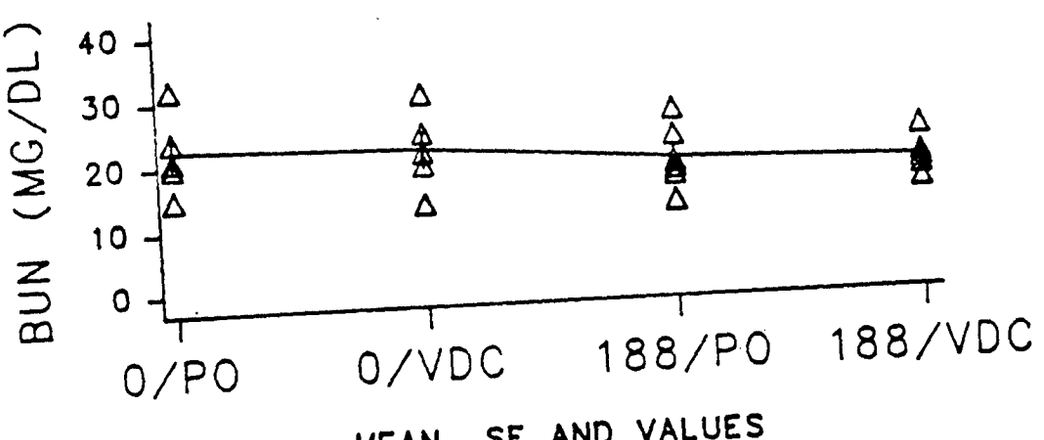
GFR, reabsorption of filtrate and urine flow rate were not affected by MCA, VDC or the combined treatment.

FEMALES

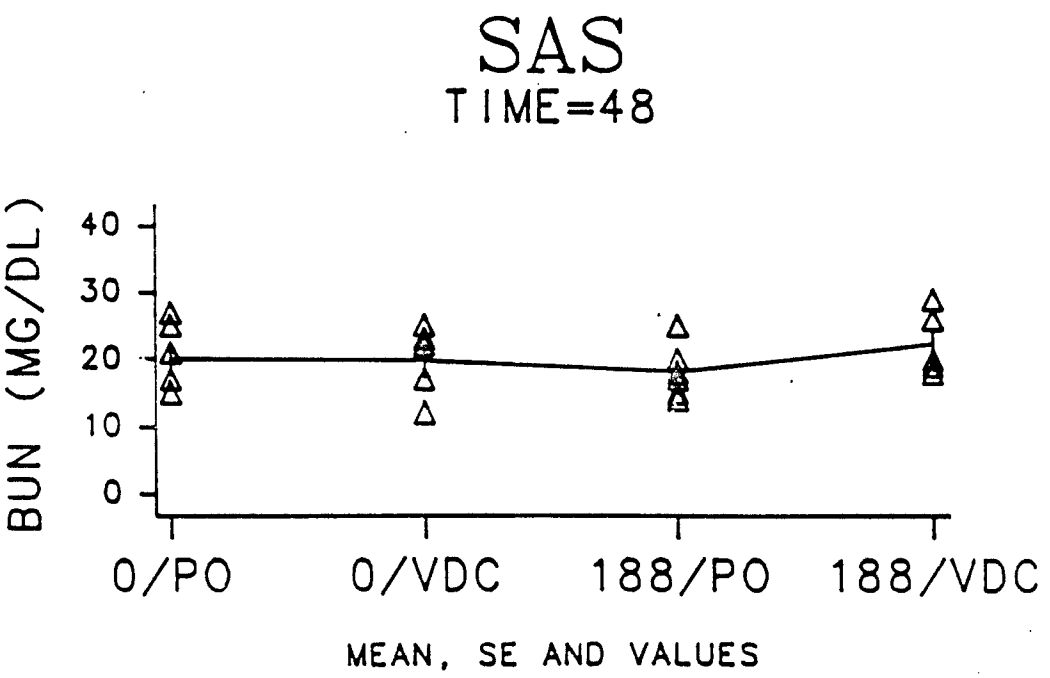
VDC decreased GFR, assessed as clearance of inulin, in both saline and MCA groups. At 24 hrs the VDC groups were approximately 50% of their control values, and BUN values were modestly, but significantly, elevated (for the saline pretreatment groups 15.6 ± 1.3 vs. 32.0 ± 6.4 and for the MCA pretreatment groups 19.0 ± 1.6 vs. 29.5 ± 1.7 mg/dl). While these results are apparently disparate, they are in agreement with the known relationship between renal function assessed by inulin clearance and lack of clearance of endogenous urea, specifically that glomerular filtration must fall below 50% of normal capacity for endogenous urea to accumulate sufficiently to be detected as being significantly elevated. The effect of VDC treatment was still apparent, and significant, at 48 hr, however the magnitude was not as great (differences between treated and control groups did not achieve statistical significance) suggesting that recovery was underway. Urine flow rate was decreased by MCA alone and by VDC in the saline pretreated group at 24 hr, by 48 hr there was no significant effect. The ability of the kidneys to reabsorb the volume of fluid filtered was not impaired at either time.

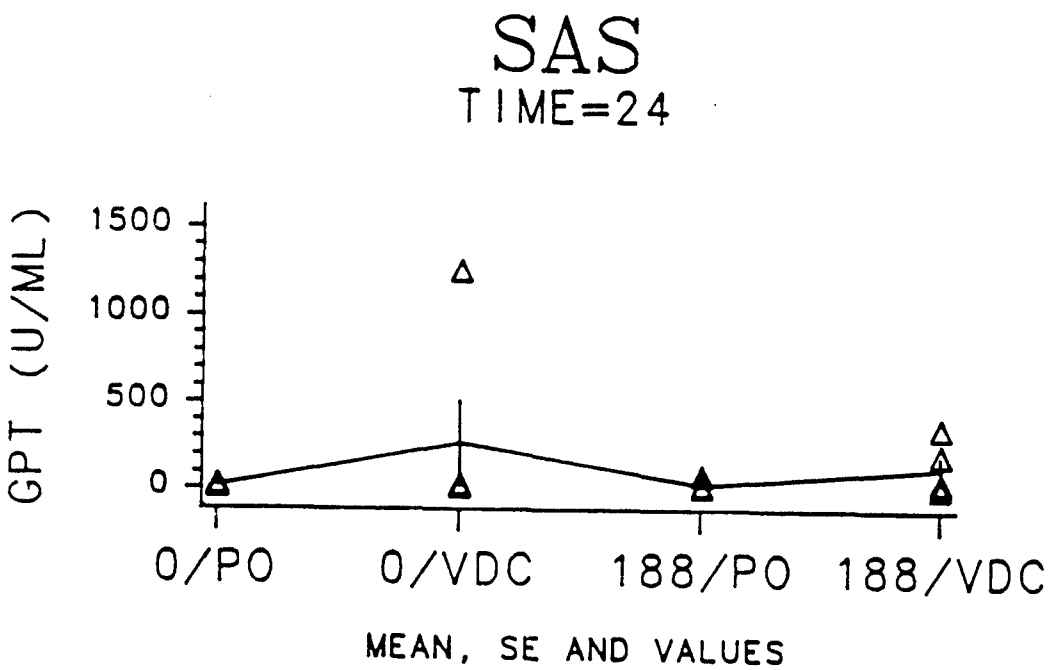
SAS

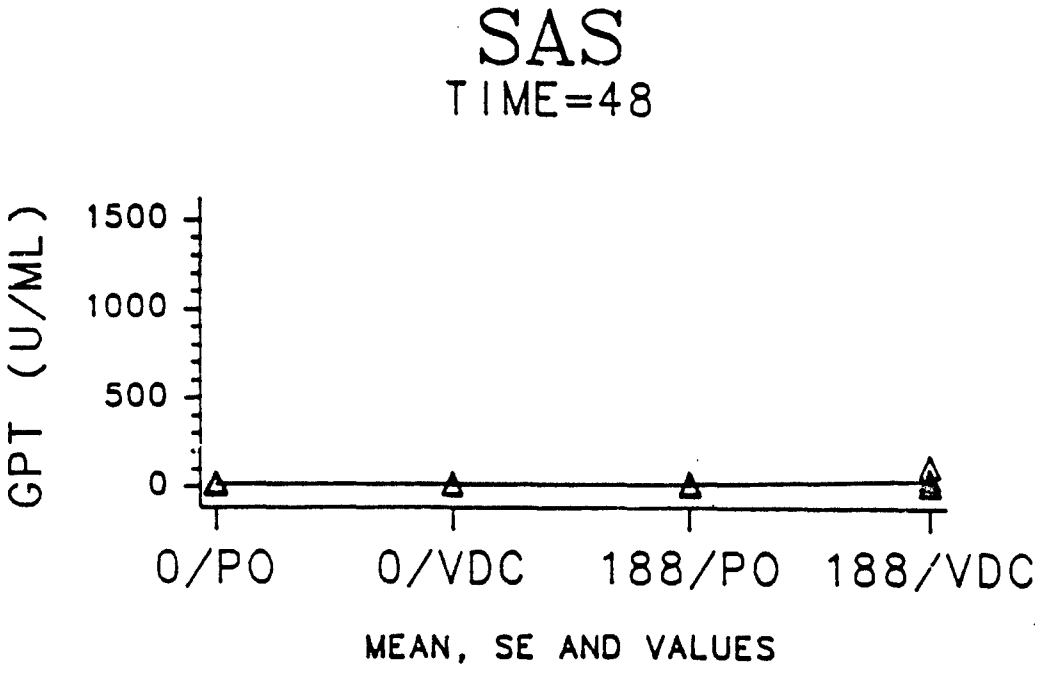
TIME=24



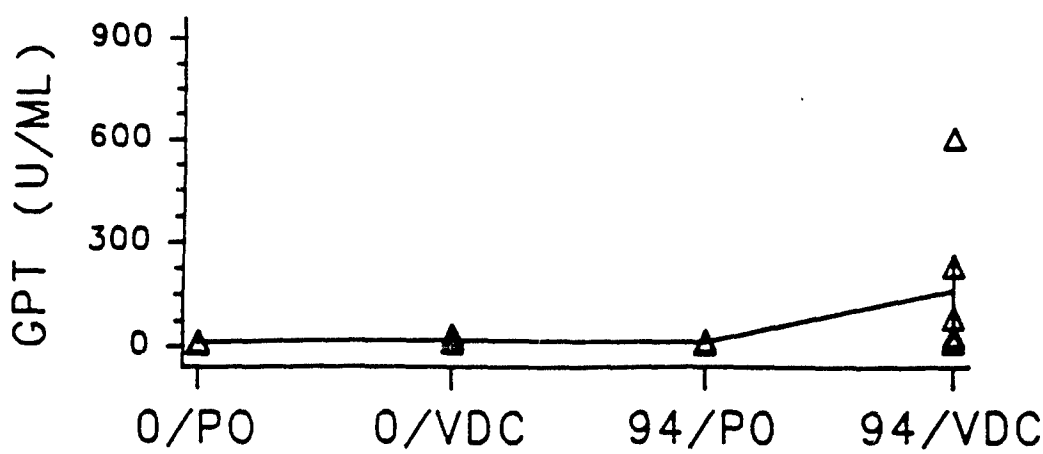
MEAN, SE AND VALUES


SAS
TIME=48
BUN (MG/DL)
40
30
20
10
0
0/PO
0/VDC
188/PO
188/VDC
MEAN, SE AND VALUES


SAS
TIME=24
GPT (U/ML)
1500
1000
500
0
0/PO
0/VDC
188/PO
188/VDC
MEAN, SE AND VALUES

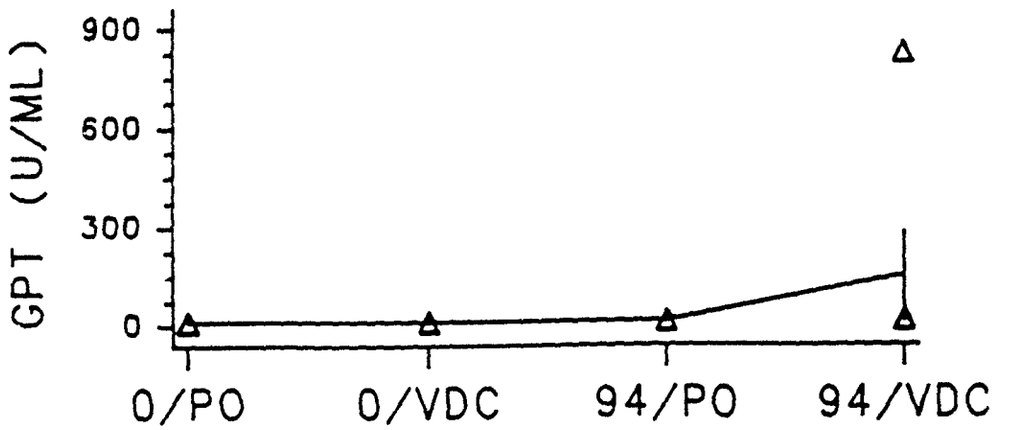

SAS
TIME=48
GPT (U/ML)
1500
1000
500
0
0/PO
0/VDC
188/PO
188/VDC
MEAN, SE AND VALUES

FEMALES
TIME=24

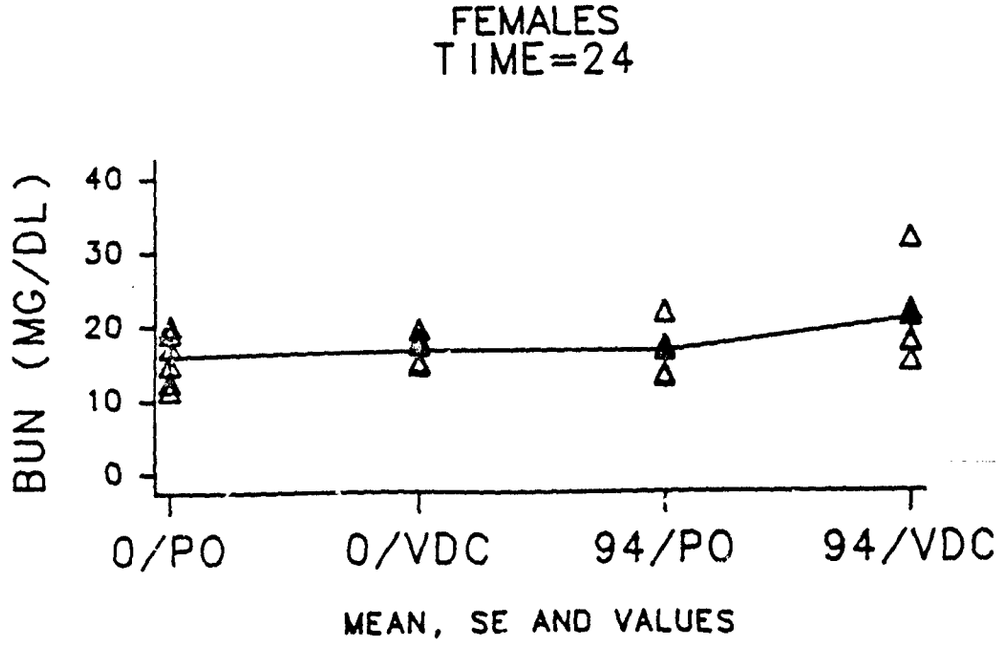


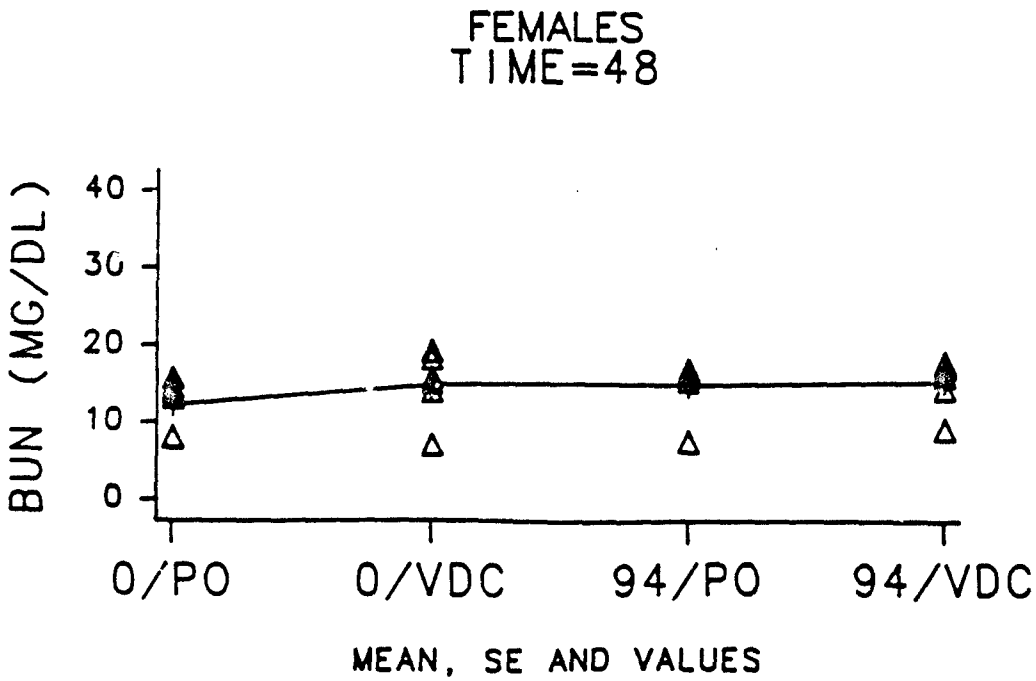
MEAN, SE AND VALUES

FEMALES
TIME=48

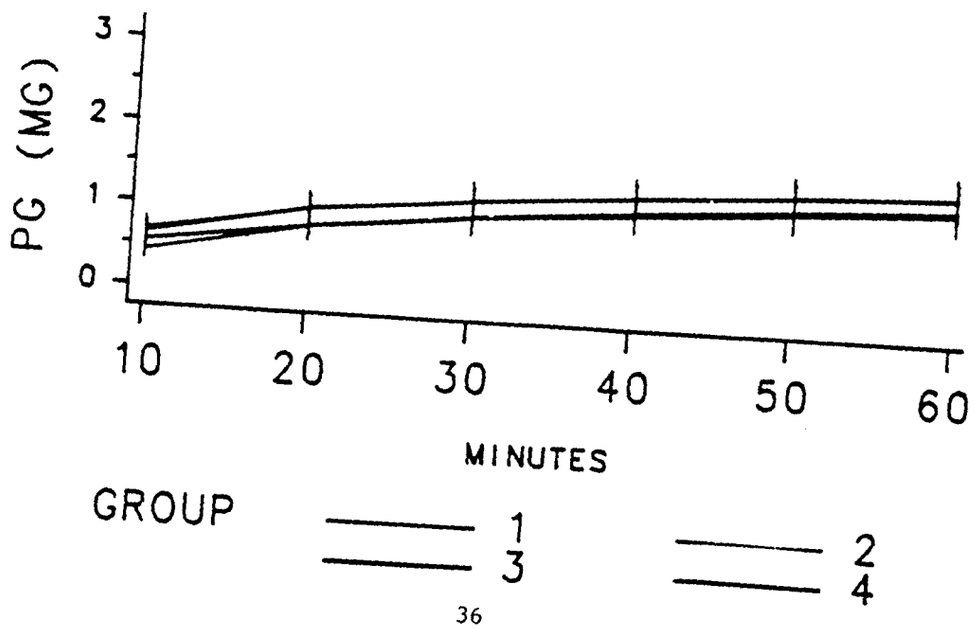


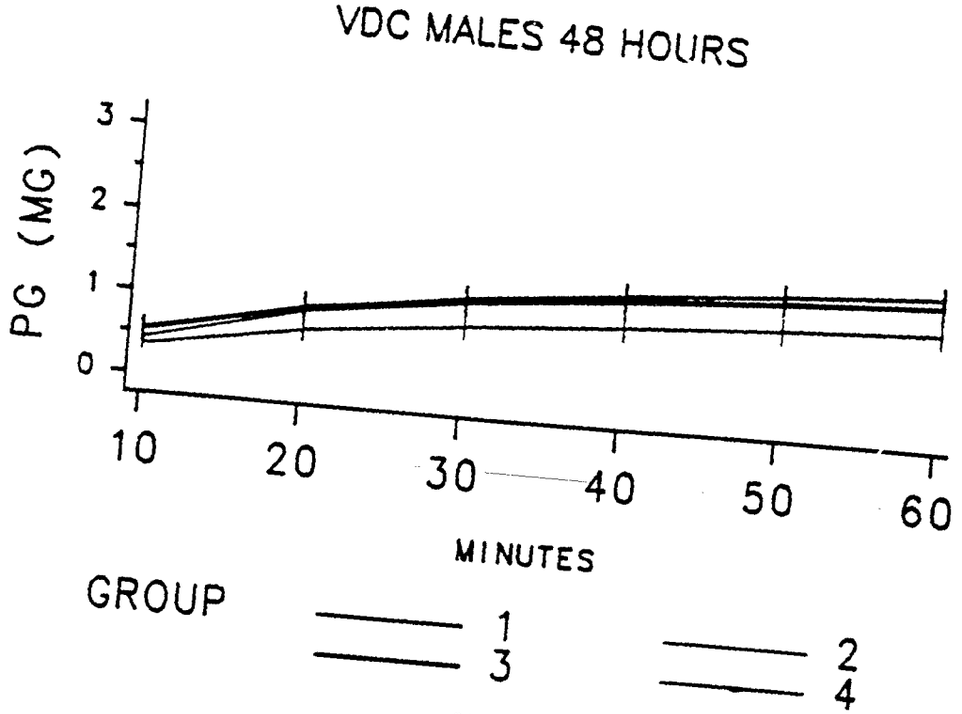
MEAN, SE AND VALUES


FEMALES
TIME=24
BUN (MG/DL)
40
30
20
10
0
0/PO
0/VDC
94/PO
94/VDC
MEAN, SE AND VALUES

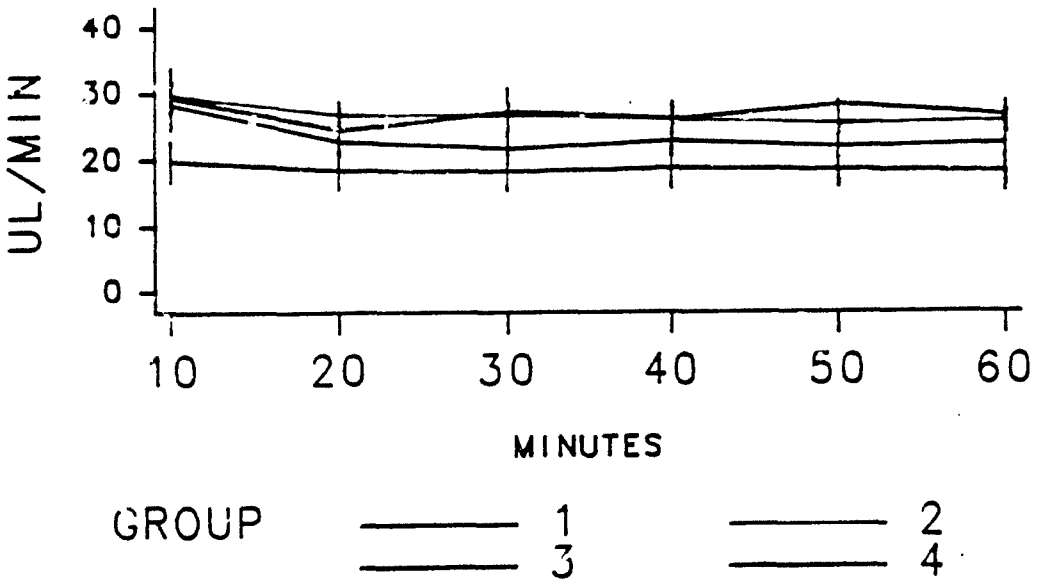

FEMALES
TIME=48
BUN (MG/DL)
40
30
20
10
0
0/PO
0/VDC
94/PO
94/VDC
MEAN, SE AND VALUES

VDC MALES 24 HOURS

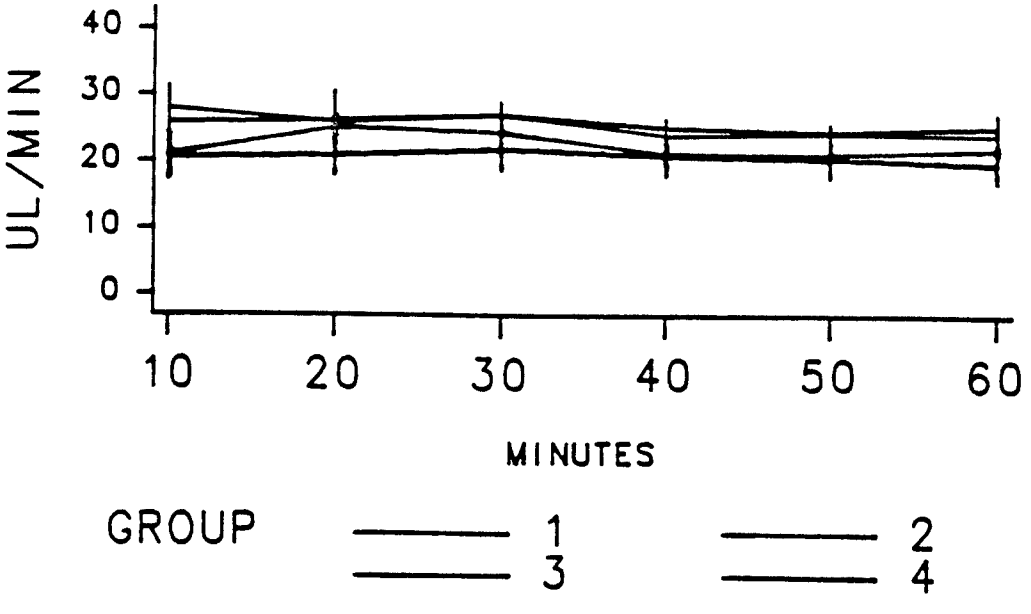



VDC MALES 48 HOURS
PG (MG)
3
2
1
0
10
20
30
40
50
60
MINUTES
GROUP
1
3
2
4

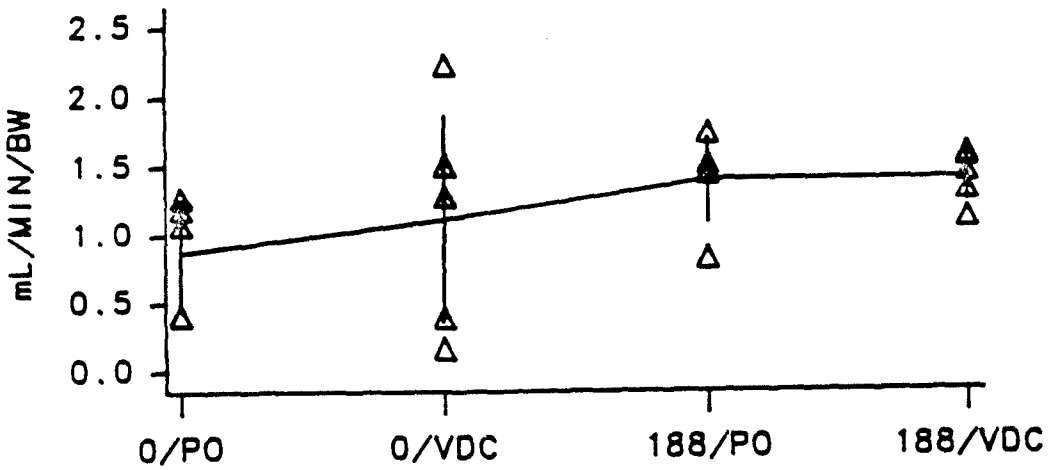
VDC MALES 24 HOURS



VDC MALES 48 HOURS

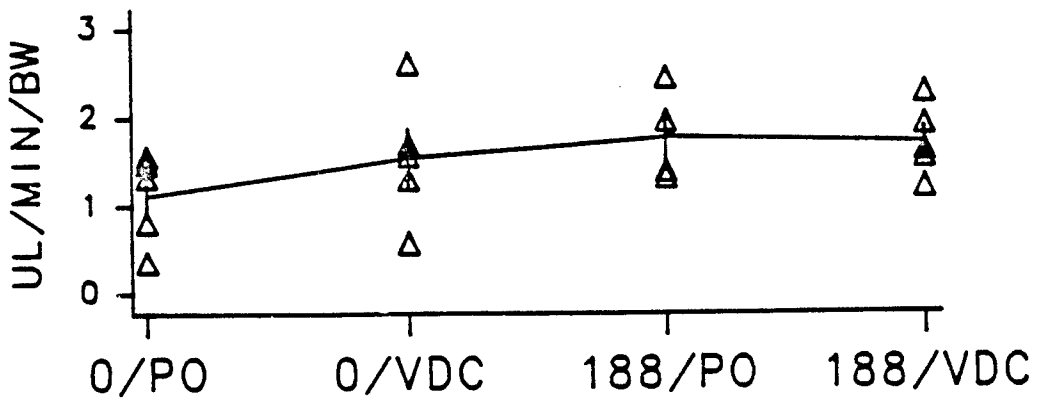


MALES 24 HRS



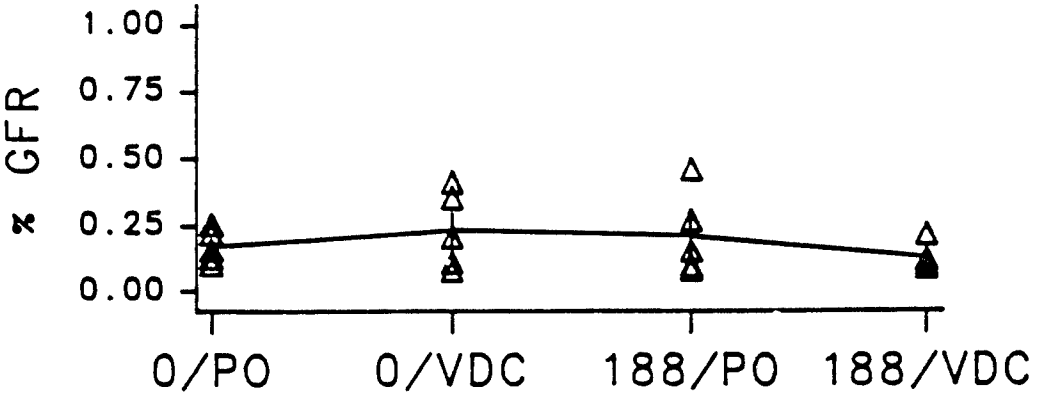
GFR (Mean, SE and Values)

MALES 24 HRS



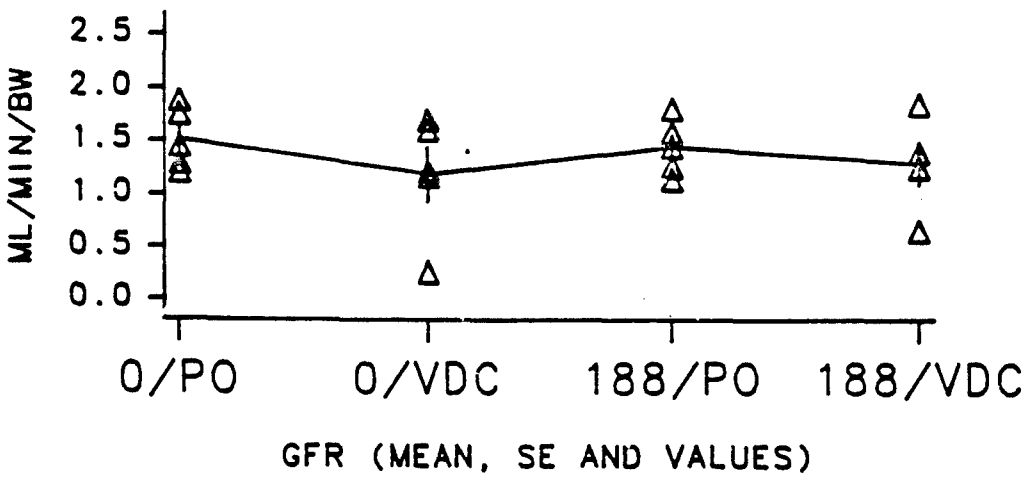
URINE (MEAN, SE AND VALUES)

MALES 24 HRS



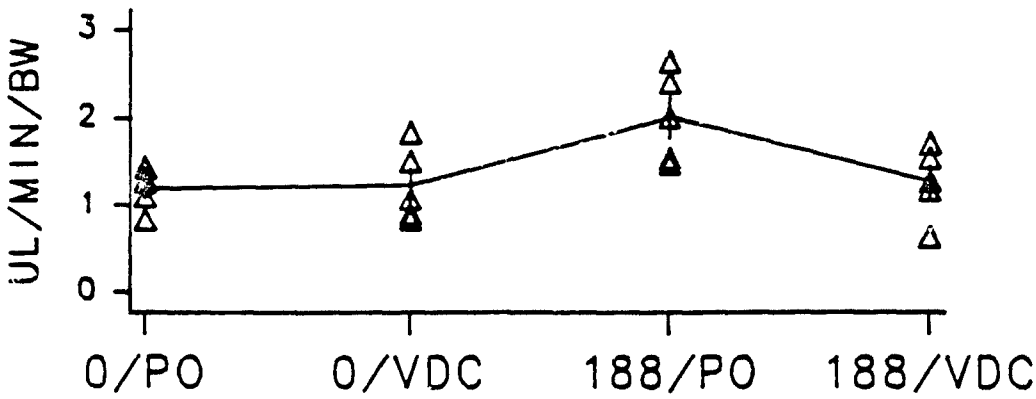
REABSORPTION (MEAN, SE AND VALUES)

MALES 48 HRS



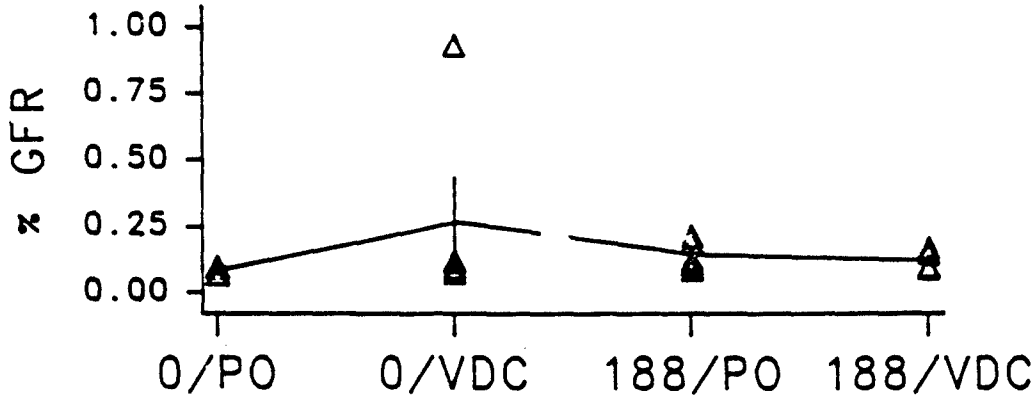
GFR (MEAN, SE AND VALUES)

MALES 48 HRS

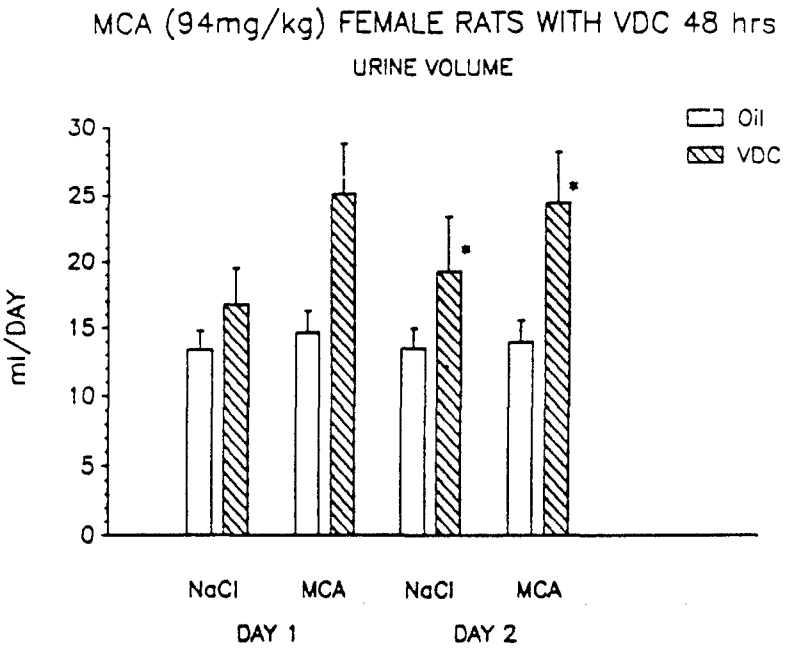


URINE (MEAN, SE AND VALUES)

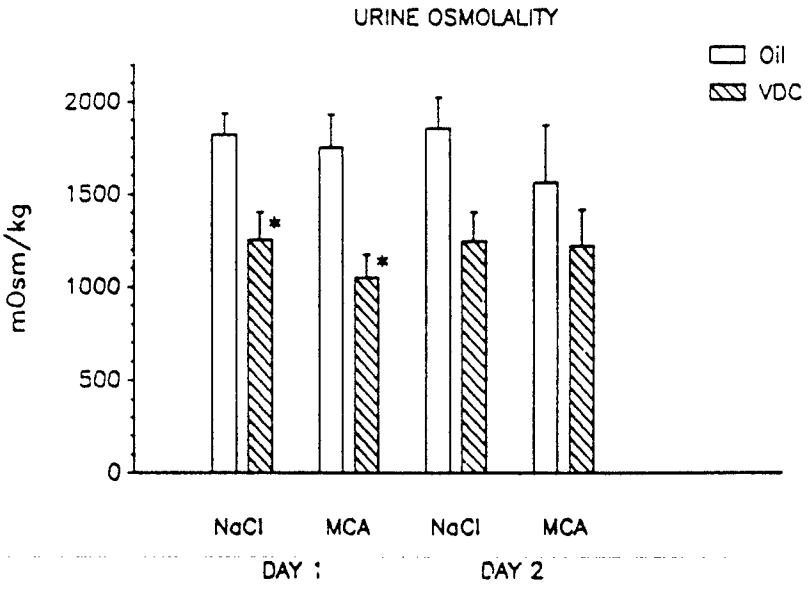
MALES 48 HRS

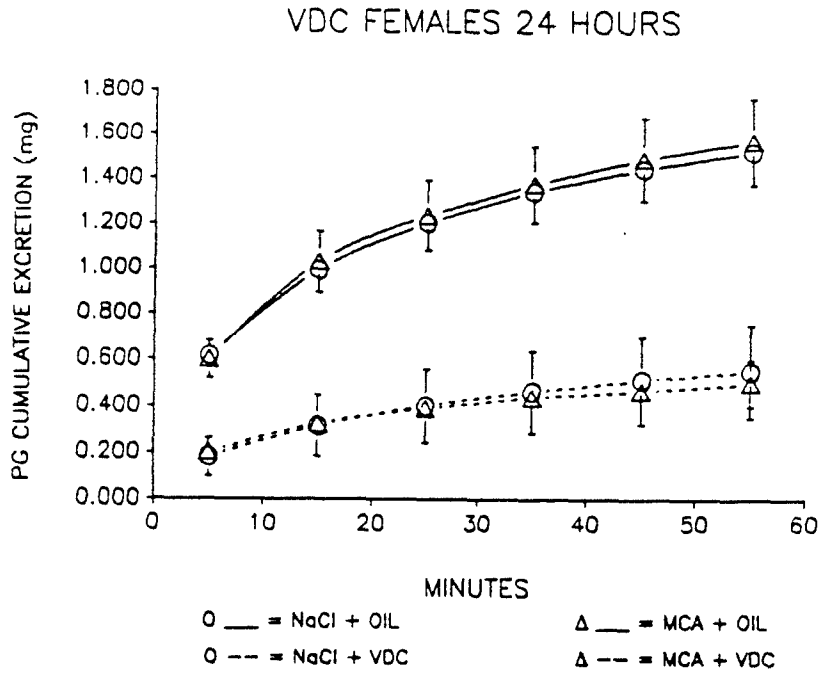


REABSORPTION (MEAN, SE AND VALUES)

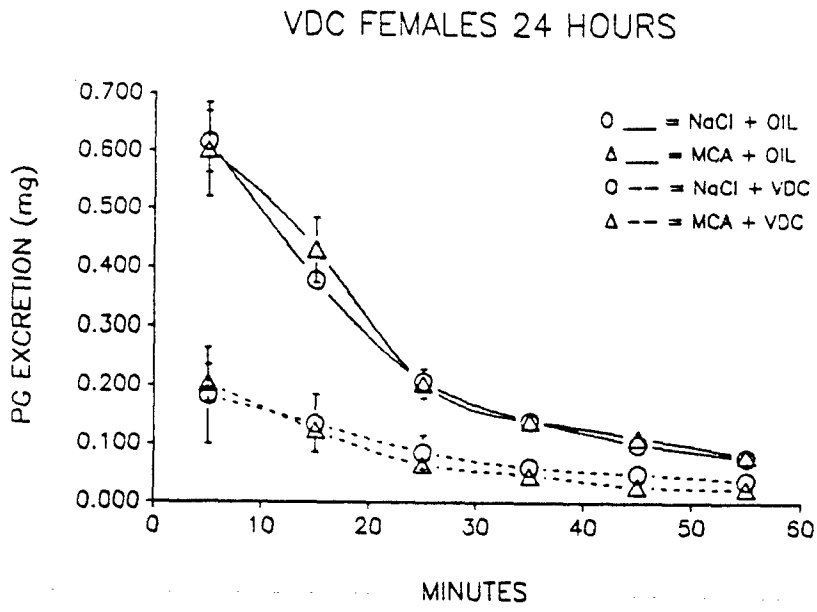

MCA (94mg/kg) FEMALE RATS WITH VDC 48 hrs
URINE VOLUME
Oil
VDC
ml/DAY
30
25
20
15
10
5
0
*
*
NaCl
MCA
NaCl
MCA
DAY 1
DAY 2

MCA (94mg/kg) FEMALE RATS WITH VDC 48 hrs

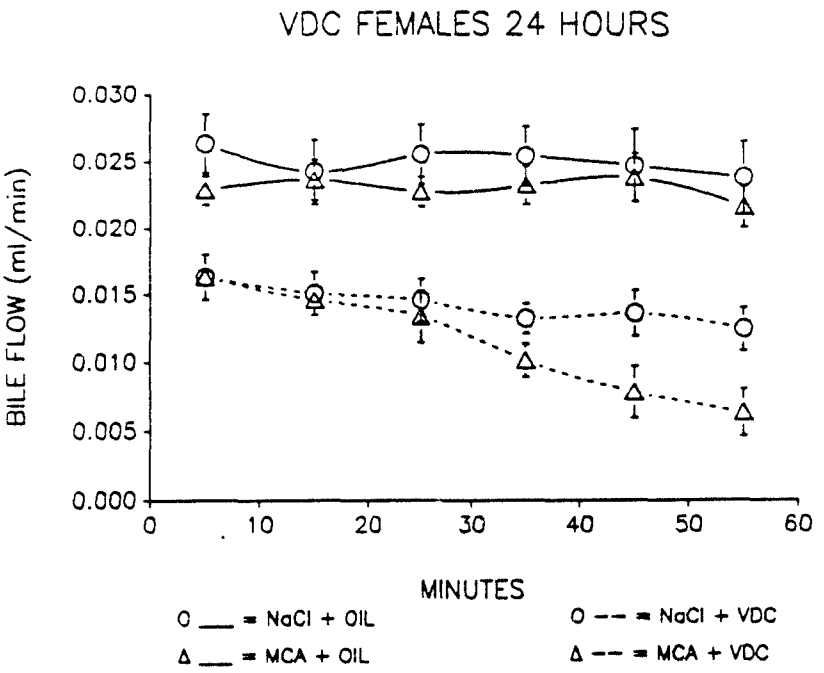




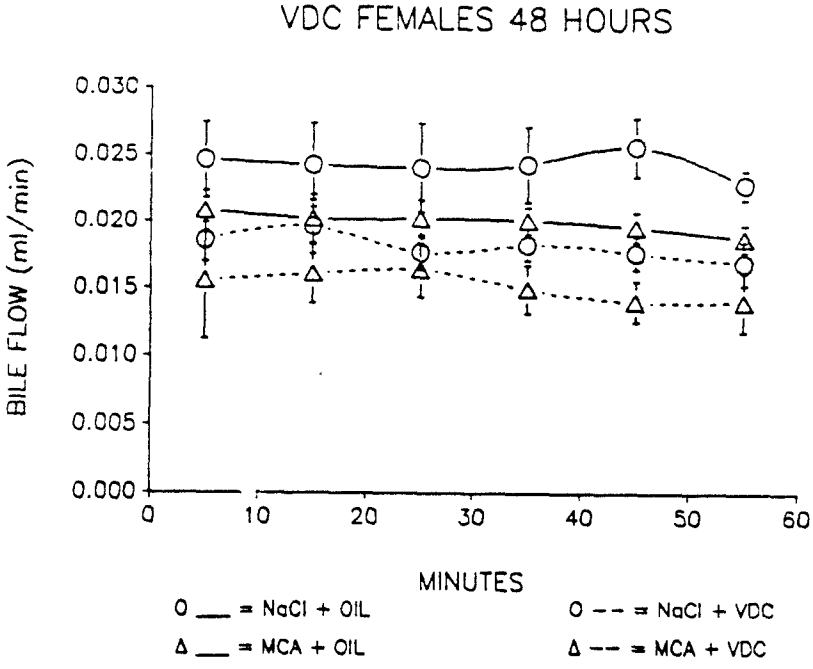
(MCA = 94mg/kg VDC = 200 mg/kg)



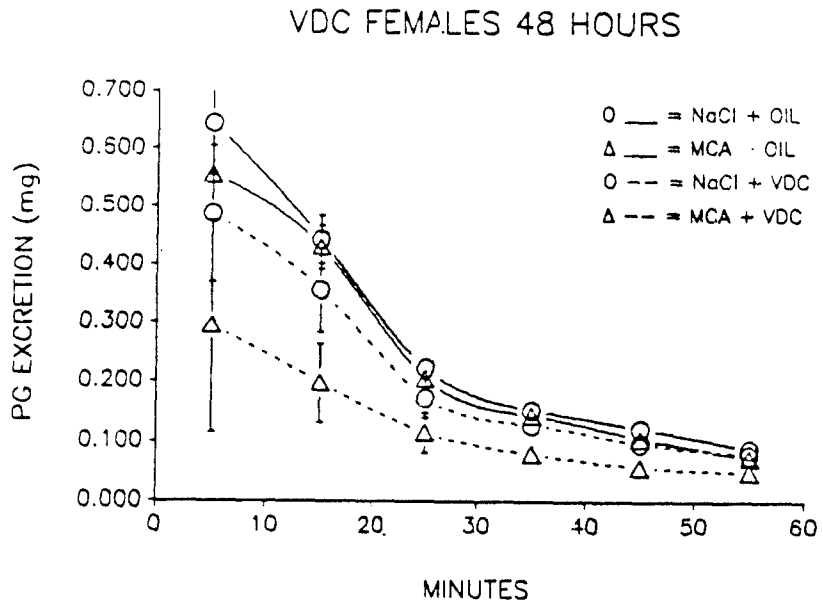
(MCA = 94mg/kg VDC = 200 mg/kg)



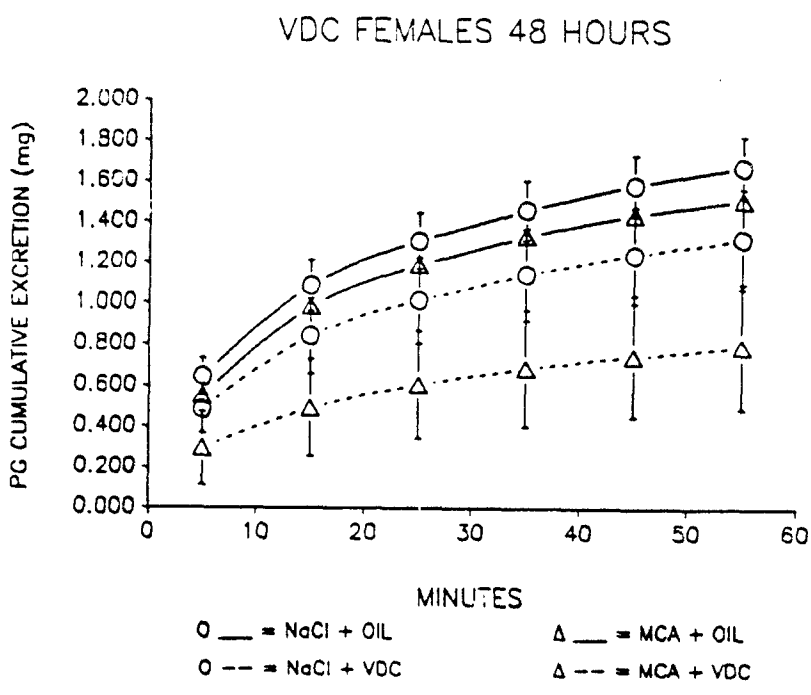
(MCA = 94 mg/kg VDC = 200 mg/kg)



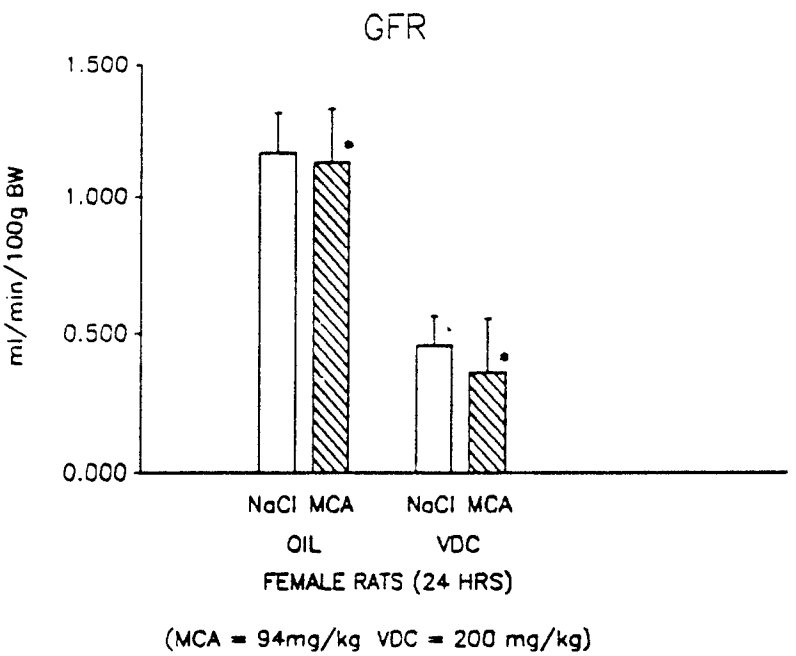
(MCA = 94 mg/kg VDC = 200 mg/kg)



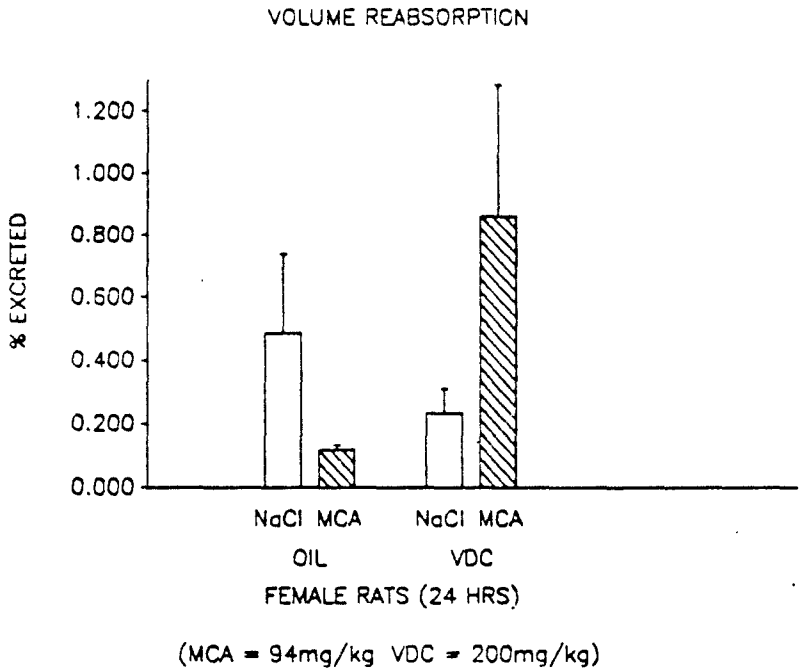
(MCA = 94mg/kg VDC = 200mg/kg)



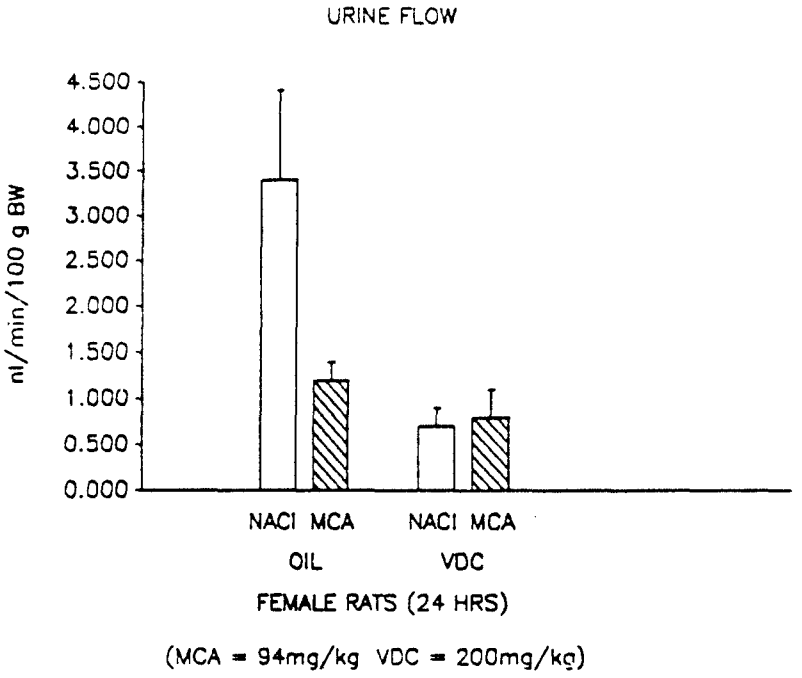
(MCA = 94mg/kg VDC = 200 mg/kg)

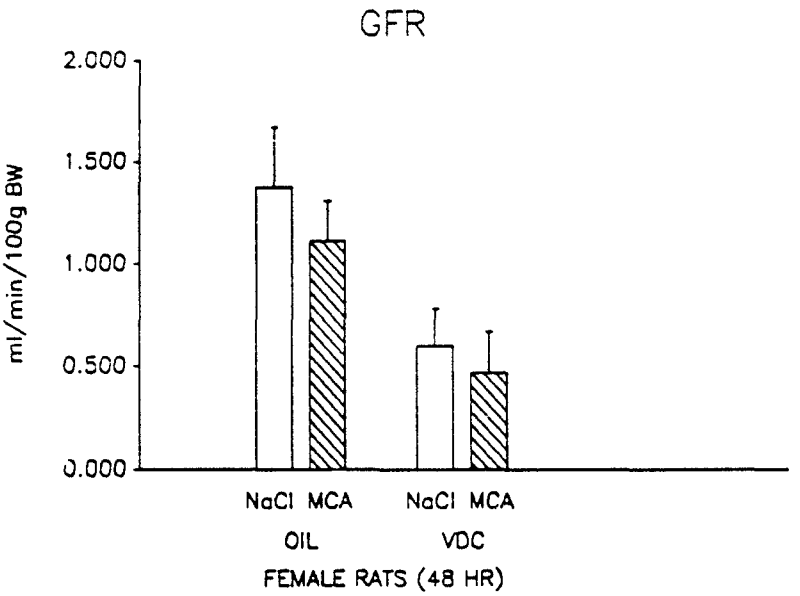


(MCA = 94mg/kg VDC = 200 mg/kg)

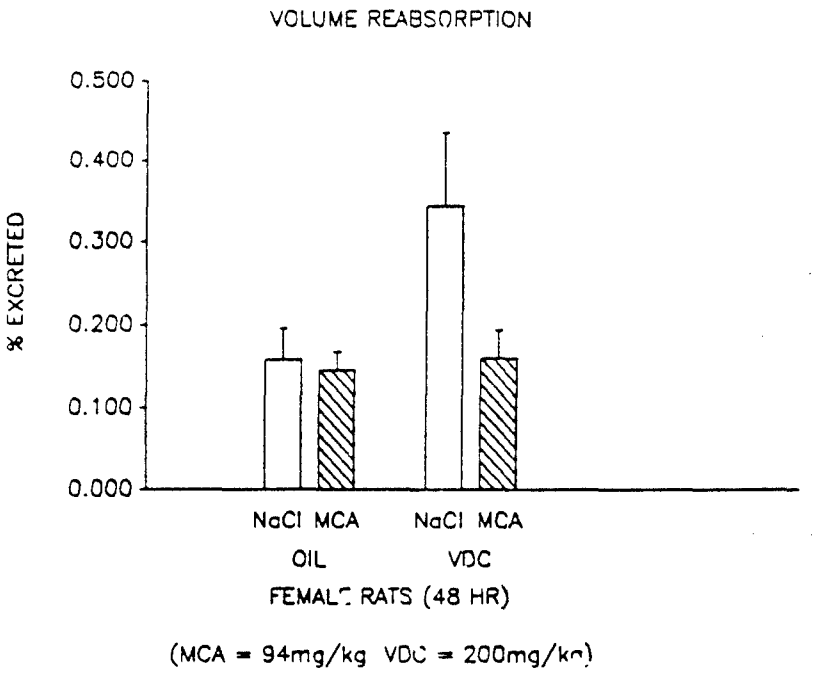


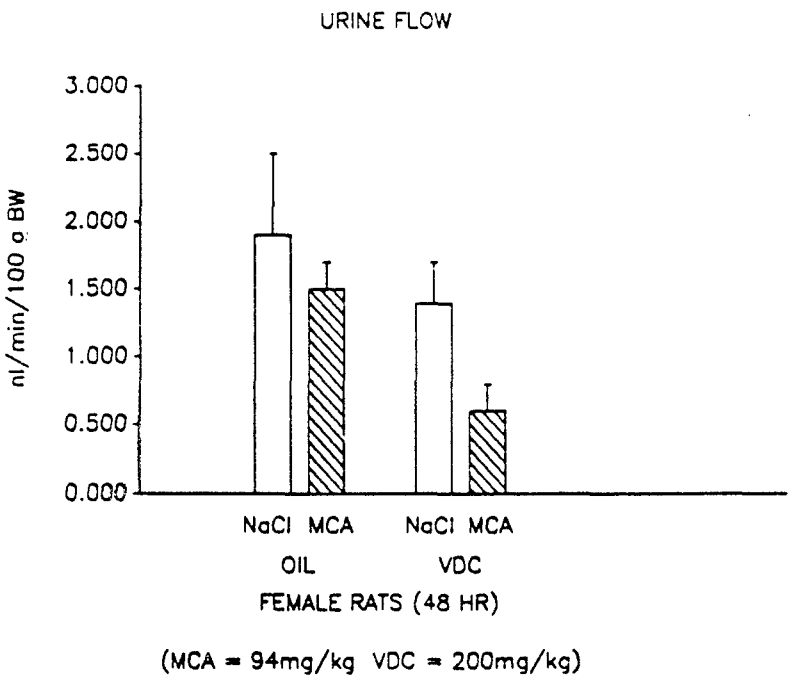
(MCA = 94mg/kg VDC = 200mg/kg)


URINE FLOW
4.500
4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
nl/min/100 g BW
NACl MCA
OIL
NACl MCA
VDC
FEMALE RATS (24 HRS)
(MCA = 94mg/kg VDC = 200mg/kg)



(MCA = 94mg/kg VDC = 200 mg/kg)


VOLUME REABSORPTION
0.500
0.400
0.300
0.200
0.100
0.000
% EXCRETED
NaCl MCA
OIL
NaCl MCA
VDC
FEMALE RATS (48 HR)
(MCA = 94mg/kg VDC = 200mg/kg)


URINE FLOW
3.000
2.500
2.000
1.500
1.000
0.500
0.000
nl/min/100 g BW
NaCl MCA
OIL
NaCl MCA
VDC
FEMALE RATS (48 HR)
(MCA = 94mg/kg VDC = 200mg/kg)

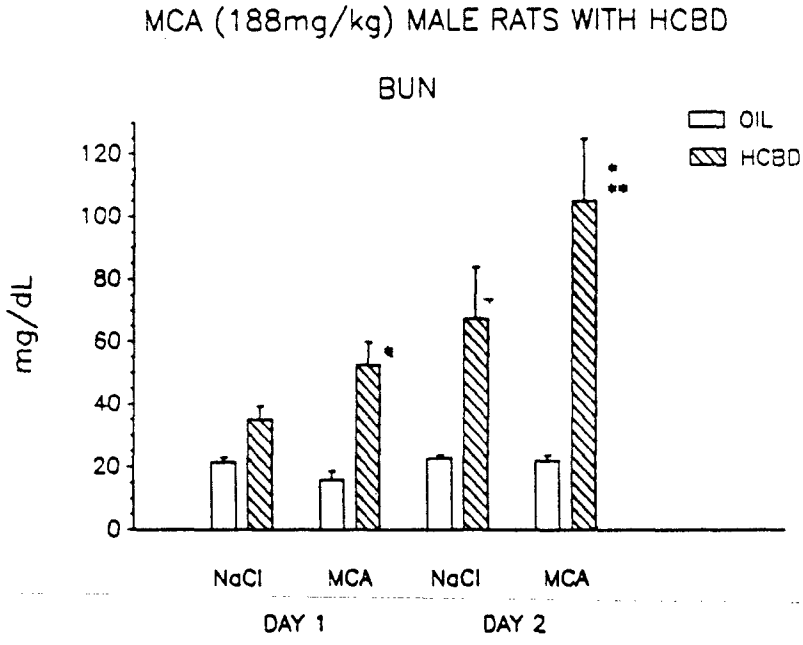
Plasma, Urine and Tissue Parameters

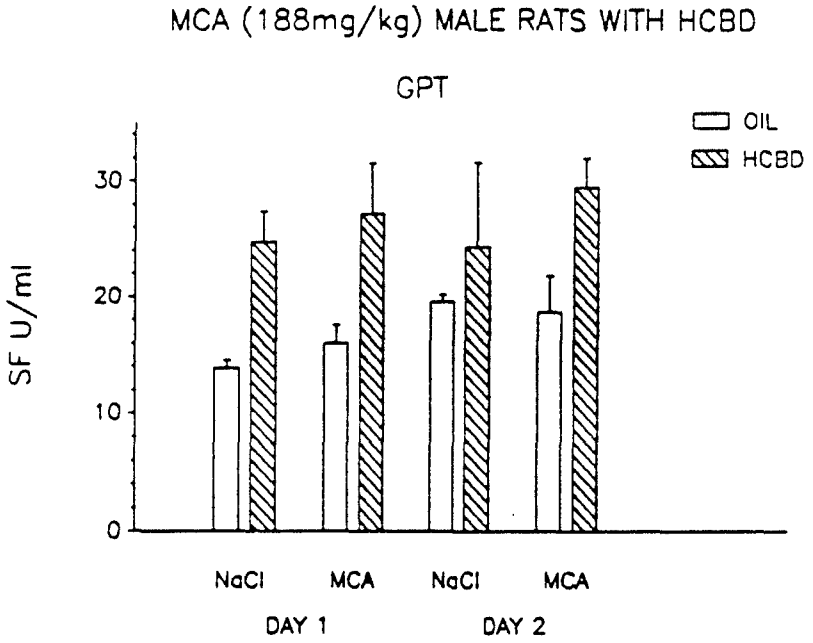
MALES

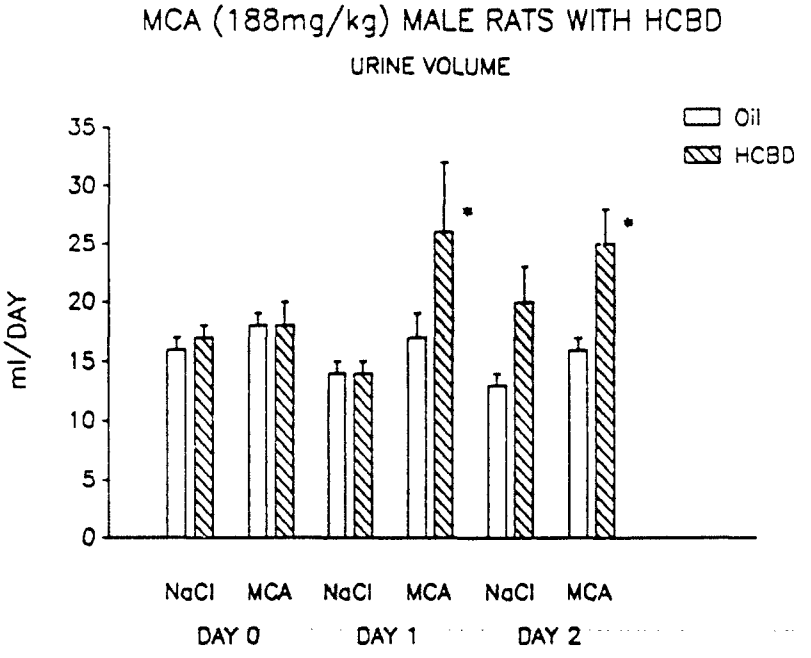
BUN was affected by treatment with HCBD (200 mg/kg) and the interaction between MCA and HCBD and the time after treatment. The HCBD groups were significantly greater than their respective oil treated controls only at 48 hr after HCBD, and significantly greater increases of BUN were found in the MCA pretreated group. GPT was somewhat increased in all of the HCBD groups, however none were significantly different from their controls. BUN was not increased after a lower dose of HCBD (50 mg/kg) however polyuria was seen in the MCA pretreated (26 ± 6 vs. 17 ± 2 ml/d) but not in the saline controls (14 ± 1 vs. 14 ± 1); both groups had nonsignificantly elevated urine output two days after HCBD. Their food consumption was decreased only the first day after HCBD. The effects on urine glucose concentration were not significant but are interesting in that MCA appears to potentiate the modest effect of the lower dose of HCBD.

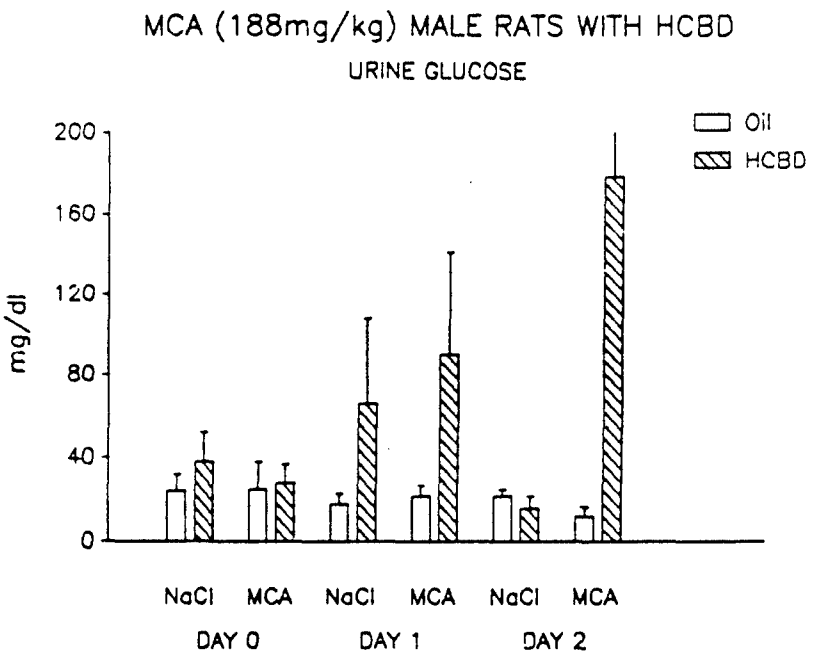
FEMALES

The lower dose of HCBD caused elevations of BUN in both saline control (60 ± 4 vs. 22 ± 1 mg/dl) and MCA pretreated (43 ± 7 vs. 19 ± 2 mg/dl) groups. In contrast to males, females had increased output of dilute urine both days after treatment with HCBD regardless of MCA pretreatment. Their water consumption was unaffected whereas their food consumption was decreased. Glucosuria was also found in both groups, and appeared to recover somewhat in the MCA group but not in the saline controls.


MCA (188mg/kg) MALE RATS WITH HCBD
BUN
OIL
HCBD
mg/dL
120
100
80
60
40
20
0
NaCl
MCA
NaCl
MCA
DAY 1
DAY 2


MCA (188mg/kg) MALE RATS WITH HCBD
GPT
OIL
HCBD
SF U/ml
30
20
10
0
NaCl
MCA
NaCl
MCA
DAY 1
DAY 2


MCA (188mg/kg) MALE RATS WITH HCBD
URINE VOLUME
Oil
HCBD
ml/DAY
35
30
25
20
15
10
5
0
*
*
NaCl
MCA
NaCl
MCA
NaCl
MCA
DAY 0
DAY 1
DAY 2


MCA (188mg/kg) MALE RATS WITH HCBD
URINE GLUCOSE
Oil
HCBD
mg/dl
200
160
120
80
40
0
NaCl
MCA
NaCl
MCA
NaCl
MCA
DAY 0
DAY 1
DAY 2